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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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CONTENTS OF VOLUME XXXII.

No. 1. January, 1917.

PAGE

Plough, Harold H. *Cytoplasmic Structures in the Male Germ Cells of Rhomaleum micropterum Beauv* 1

Wilder, Inez W. *On the Breeding Habits of Desmognathus fusca* 13

Goldfarb, A. J. *The Symmetry of Grafted Eggs in Relation to Giant Larvæ Formation in Arbacia punctulata* 21

Goldschmidt, Richard. *On a Case of Facultative Parthenogenesis in the Gypsy Moth Lymantria dispar L. With a Discussion of the Relation of Parthenogenesis to Sex* 35

No. 2. February, 1917.

Schaeffer, Asa A. *Reactions of Ameba to Light and the Effect of Light on Feeding* 45

Child, C. M. *Susceptibility Gradients in the Hairs of Certain Marine Algæ* 75

Allee, W. C. *The Salt Content of Natural Waters in Relation to Rheotaxis in Asellus* 93

Weese, A. O. *An Experimental Study of the Reactions of the Horned Lizard, Phrynosoma modestum Gir., A Reptile of the Semi-desert* 98

No. 3. March, 1917.

Allen, Bennet M. *Effects of the Extirpation of the Anterior Lobe of the Hypophysis of Rana pipiens* 117

Lillie, Ralph S. *Temperature-Coefficients in the Activation of Starfish Eggs in Butyric Acid* 131

Hamilton, Clyde C. *The Behavior of some Soil Insects in Gradients of Evaporating Power of Air, Carbon Dioxide and Ammonia* 159

Chenoweth, Homer E. *The Reactions of Certain Moist Forest Mammals to Air Conditions and its Bearing on Problems of Mammalian Distribution* 183

Harris, J. Arthur, and Lawrence, John V. *The Osmotic Concentration of the Sap of the Leaves of Mangrove Trees* 202

No. 4. April, 1917.

Child, C. M. *Experimental Alteration of the Axial Gradient in the Alga, Griffithsia bornetiana* 213
Clayberg, Harold D. *The Effect of Ether and Chloroform on Certain Fishes* 234
Richards, A. *The History of the Chromosomal Vesicles in Fundulus and the Theory of Genetic Continuity of Chromosomes* 249

No. 5. May, 1917.

Patterson, J. T. *Studies on the Biology of Paracopidosomopsis. I., Data on the Sexes* 291
Newman, H. H. *On the Production of Monsters by Hybridization* 306
Foot, Katharine, and Strobell, E. C. *Results of Crossing Euschistus variolarius and Euschistus ictericus with Reference to the Inheritance of two Exclusively Male Characters* 322

No. 6. June, 1917.

Hickernell, Louis Max. *A Study of Desiccation in the Rotifer, Philodina roseola, with Special Reference to Cytological Changes Accompanying Desiccation* 343
Shaffer, E. L. *Mitochondria and Other Cytoplasmic Structures in the Spermatogenesis of Passalus cornutus* 407

Vol. XXXII. *January, 1917.* *No. 1.*

BIOLOGICAL BULLETIN

CYTOPLASMIC STRUCTURES IN THE MALE GERM CELLS OF RHOMALEUM MICROPTERUM BEAUV.

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In a recent paper Lewis and Robertson gave an account of certain cytoplasmic structures in the male germ cells of *Chorthippus curtipennis* Scud. as seen by the tissue culture method. It is the purpose of this paper to give somewhat similar data for the Florida lubber grasshopper, *Rhomaleum micropterum* Beauv., on the basis of both fixed and living material. My observations were made mainly in order to determine the origin and significance of the chromatoid body. They closely parallel those of the previous authors, and may perhaps clear up some doubtful points.

While studying the maturation phenomena in *Rhomaleum* in 1914 it was found that a small round densely staining body was always present in the cytoplasm of the cells of the late growth period. Further study disclosed the fact that this body appeared in the very early growth period, enlarged up to the stage of diakinesis, and at both the first and second maturation divisions passed unchanged into one of the two daughter cells. During the metamorphosis of the spermatid this body passed gradually down into the tail region of the future spermatozoon, and was eventually cast off and degenerated in the lower end of the follicles. This history is exactly parallel to that of a similar but larger body described by Dr. E. B. Wilson ('13) in the spermatogenesis of the hemipter *Pentatoma senilis*, and called by him the "chromatoid body." It is now known that a granule or granules of similar behavior are present in the developing male germ cells of the following forms: horse, pig, bull, rabbit, crayfish and probably in the rat, mouse, and a number of Hempitera. It seems probable therefore that the appearance and elimination of such material from the spermatozoa is a fairly general phenomenon throughout the animal kingdom.

The recent study of Lewis and Robertson on the living germ cells of *Chorthippus curtipennis* showed that certain granules were present in the spermatogonia and passed on somewhat enlarged into the metamorphosing spermatids. From the fact that these granules stained with neutral red in contrast to the mitochondria they were called "neutral red granules." Up to the time their paper was published the work with *Rhomaleum* had been carried exclusively with fixed material, but in view of the similarity of behavior between these granules and the chromatoid body in the Florida grasshopper it was believed that they were of similar material and significance. Work on the living male germ cells of *Rhomaleum* during the past summer has confirmed this belief in every respect, and has made it possible to add a few facts to the data of the previous authors.

The material from which the observations were made was secured from the Supply Department at Woods Hole during the summers of 1915 and 1916. That used for permanent preparations was fixed in the usual fixatives including the modified Flemming's fluid used for the Benda mitochondrial technique. A large number of different staining methods were employed including the Auerbach mixture, the Borel stain, together with the Altmann acid fuchsin stain and the alizarin-crystal violet combination of Benda for mitochondria. The osmic mixtures were invariably the best cytoplasmic fixatives and from material so treated all of the drawings from permanent preparations were made. The methods used with the living material were for the most part those of Lewis and Robertson. Intravitam staining with janus green B and neutral red was employed, the stains being used both separately and together. The culture medium used was the modified Locke's solution of these authors made up with sea water, though some follicles were stained in Ringer's solution with fair success. The tissue cultures were only partially successful, most of the cells being abnormal after thirty-six hours. This was probably due to lack of familiarity with the technique. Ample data for the purposes of this paper were secured, however, since cells can be secured at any desired stage without watching any particular cell for any great length of time.

Observations.

However we may interpret the behavior of the chromosomes, *Rhomaleum* corresponds in all essentials with the account of orthopteran spermatogenesis given by Davis ('08) for *Dissosteira*, with the *Hippiscus* type of the Acrididæ as described by McClung ('14) and with *Phrynotettix* (Wenrich, '16). A connected account of the stages through which the chromosomes of *Rhomaleum* pass during the growth period would therefore correspond in most respects with that given by Davis.

In the spermatogonia the cytoplasm in fixed material is comparatively clear except for a minute fibrillar network. In addition several minute but darkly staining granules can usually be seen scattered about the cytoplasm. Mitochondria are apparently never visible in the spermatogonia even when the material is fixed in the modified Flemming and stained with the so-called mitochondrial stains. In living material however the cytoplasm shows no fibrillar network, but the mitochondria appear irregularly scattered about the cytoplasm or more often in a fairly dense mass close to the nuclear wall. In the resting stages in *Rhomaleum* they are very delicate granules, staining brilliantly with janus green, and show very little tendency toward any thread-like arrangement. No dividing primary spermatogonial cell was observed. In the resting stages of the secondary spermatogonia the mitochondria apparently have a tendency to be less scattered and more densely crowded next to the nuclear wall (Fig. 1). In dividing secondary spermatogonial cells the mass of granules is arranged in irregular rows about the spindle, and fairly evenly divided without assuming any definite thread-like form. The granules visible in the fixed material can also be seen in the living cells. They are usually five or six in number, and do not stain with janus green. After being treated with neutral red for about an hour, however, they appear faintly pink. The number is often as large in the secondary as in the primary spermatogonia, and this gives some reason for supposing that new granules of this sort are being gradually formed. While these granules cannot be followed during the spermatogonial divisions there seems little doubt that they lie inert in the cell and their distribution is merely hit or miss to one or the other of the daughter cells.

After the last spermatogonial telophase the nuclei enter on the stage shown in Fig. 2, in which the chromatin appears as a light network in prepared material. This network shortly becomes aggregated into flocculent masses corresponding roughly in number to the diploid number of the chromosomes. This "massive body" stage is beginning to appear in Fig. 3 and Fig. 4. The mitochondria in these early stages of the growth period are extremely difficult to demonstrate in fixed material—as indeed they are throughout the spermatogenesis—even by the special technique for mitochondria. At times, however, a cloudy mass can be seen forming a cap over one side of the nucleus (Fig. 2). With janus green, however, they become very prominent in the living material. The granules are larger and seemingly in greater numbers than in the spermatogonia. The details of their behavior are similar to those in *Chorthippus*, except that here again there is no tendency to assume the thread-like form.

Throughout these earlier stages of the growth period a mitosome, the remains of the last spermatogonial division, is present. Often an actual bridge between the two daughter cells persists for a short time after the division is complete. This is the condition seen in Fig. 3. The mitosome rapidly disappears after the stage shown in Fig. 3, and is never found after the "massive body" stage. At about this time there appears in about the center of the cytoplasmic mass a more or less definite sphere which is browned by osmic and only slightly stained by hæmatoxylin. At first I took this to be a mitosome, which it strongly resembles, but later it was found that a definite mitosome was present at the same time, as shown in Fig. 3. The sphere is shown with great clearness in Fig. 4, where it lies in a large vacuole—probably due to imperfect fixation—which is surrounded by masses of mitochondria. This sphere is not visible in the living cells, whether stained or not, but its presence can be inferred from the fact that in the growth period the mitochondria are usually in two separate masses with a clear space close to the nuclear wall between. An extreme case of this sort is shown in a cell in the bouquet stage (Fig. 6) which will be referred to later. Here it is very clear that a spherical mass must separate the mitochondria. This characteristic clumping of the mitochondria in two groups is mentioned by Lewis and Robertson, but no explanation is attempted. The behavior of this sphere shows that it is in the nature of an attraction sphere or idiozome (Meves).

Finally there are in the cytoplasm of these cells of the early growth period from three to six or more of the "neutral red granules" mentioned above. They are larger and more prominent than in the spermatogonia, and are shown stained with hæmatoxylin in Figs. 2 and 3. They may lie anywhere in the cytoplasm, but are more often among the mitochondria and close to the idiozome. In the living material they do not appear in the unstained cells, but when cells are stained with neutral red, they take the stain faintly some time after treatment. There are then four sorts of inclusions in the cytoplasm of the cells in the early stages of the growth period: (1) the mitosome, (2) the idiozome, (3) the fragments called "neutral red granules," (4) the masses of granular mitochondria.

From the stage of the massive bodies the chromosomes uncoil into the leptotene condition. The threads are fine and at first appear as a tangled mass. The monosome is of course an exception to this rule for it remains as a heavy densely staining mass. Shortly after the leptotenes begin to suggest a polarization, the threads become doubled and pass rapidly into the diplotene condition. Here they remain until the growth period is completed and diakinesis begins. In this process of polarization the spherical idiozome in the cytoplasm apparently plays an important part. It is not always visible in the stained material even when the preparations are carefully extracted with a view to making it clear, but in *every case* in which it can be seen in the late leptotene or diplotene stages it is found that the *chromatin threads are polarized towards it.* Two clear cases of polarized diplotenes are shown in Figs. 5 and 6. Both are approximately the same stage, the first from a fixed and mounted preparation, and the second from a living cell stained with janus green. The first shows the diplotene threads clearly polarized toward the idiozome, while the second the indefinite heavy threads polarized toward a vacant space in the cytoplasm outlined by the mitochondria. After the diplotene stage the idiozome is never visible.

The failure of previous observers to identify an attraction sphere in at least some of the Acrididæ is rather peculiar, for it seems almost certain that it has been figured before. A body of similar appearance, called by Davis a "mitochondrion," is shown in at least his Figs. 31, 34 and 42. In view of the observed behavior of the mitochondria this is of course incorrect, and it seems improbable that it can be a mitosome at the stages indicated. As has been observed above, however, the idiozome is not always visible, and when taken in conjunction with the fact that a clear polarization of the threads is sometimes hard to find, this may indicate that the idiozome is a structure which is sometimes present and sometimes not.

It remains to trace the behavior of the "neutral red granules" during these earlier stages of the growth period. As stated above and shown in Figs. 2, 3 and 4, these granules are larger than the mitochondria, stain densely with hæmatoxylin, and may lie anywhere in the greatest mass of cytoplasm. When the diplotene stage is reached (cf. Fig. 5) we find instead of several granules a single rather large spherical mass lying in a clear vacuole. It is now exactly similar, though smaller, to the chromatoid body of *Pentatoma seilis* described by Wilson. This body stains very heavily with hæmatoxylin, while in the living cell it is practically invisible. When the living material has been treated with neutral red for about an hour, however, it appears as a highly refractive pink drop in the cytoplasm (Fig. 8). Intermediate stages between these two conditions have not been found in the living material, and even in the fixed preparations one usually finds that the cells from the massive body stage on, contain a single chromatoid body in the cytoplasm. Cells are found now and then in which several granules are apparently fused in one mass, or in which two seem in the process of fusion, as in Fig. 3. It is certain at least that the single chromatoid body appears at the expense of the earlier small granules, and this fact makes it probable that they have become fused into one mass. Occasionally, however, cells appear in which a granule is present in addition to the larger chromatoid body, and I have observed one case in which two equal granules—or chromatoid bodies—were present in a cell at the time of diakinesis. The mass of the two would approximately equal that of the single body usually found at this time. It is possible therefore that the chromatoid body represents simply the enlargement of one of the smaller granules, as suggested by Wilson, who observed a few granules in the cytoplasm in the presynaptic stages of *Pentatoma*. In any case the single chromatoid body in *Rhomaleum* is undoubtedly made up of the material of the granules seen in the cytoplasm of the spermatogonia and early growth period.

Throughout the growth period the chromatoid body increases in size. When the early diakinesis is reached the cell has reached its maximum size, and at this point the chromatoid body is also largest. Fig. 7 shows a cell in which it was even larger than usual, while Fig. 8 represents a living cell at about the same stage, stained with both janus green and neutral red. The mitochondria at this stage are scattered about the cytoplasm to some extent, though they still show a tendency to be aggregated in a mass at one side of the nucleus. From this point onward the chromatoid body behaves as an inert mass, which does not increase in size, and is not affected in the slightest degree by either maturation division. At each division the mitochondria are arranged in the manner described by Lewis and Robertson, except that here again I noticed but little tendency to the thread-like form so evident in *Chorthippus*. The granules are arranged in rows about the spindle but their separate granular condition is always evident. The chromatoid body remains in whichever half of the cell the division plane happens to place it. It is sometimes within the mass of spindle fibers as in Fig. 9, which represents a first spermatocyte telpohase, but more often toward the periphery of the cell. Fig. 10 shows a very late telophase of the second maturation division. The nuclei have already assumed the flocculent appearance characteristic of the early spermatids. The cells are completely divided except for the spindle which still bridges them. The mitochondrial masses have already become aggregated into the definite spheroidal nebenkerns, and the cytoplasm is clear and free from granules. The chromatoid body, here unusually small, is seen in one of the cells close to the wall of the nebenkern.

It is unnecessary to give details of the metamorphosis of the spermatids. In living material stained with janus green Lewis and Robertson have shown that the nebenkern appears as a mottled spherical mass, which finally divides into hemispheres, between which the axial filament passes. These granular hemispheres elongate as the tail draws out, eventually forming two dotted lines, one on either side of the axial filament. Here again the mitochondria appear as separate granules, with little tendency to fusion even in the mature sperm tail. In a few preparations stained by the Benda method I have noticed the "acrosome sphere," described by Meves and by Montgomery at the opposite side of a spermatid from the nebenkern (Fig. 11). It stains purple, which is the typical mitochondrial reaction. I have been unable to trace its origin or subsequent history, but by analogy with other forms it probably forms the perforatorium of the mature sperm. Since the chromatoid body never divides it should be found in one fourth of the spermatids. In order to test this expectation ten cysts of spermatids in various stages were selected at random from several preparations, and the total number of cells and chromatoid bodies recorded. The results follow:

No. of Cyst.	No. of Spermatid Nuclei.	No. of Chromatoid Nuclei.
1	59	12
2	78	21
3	56	15
4	54	11
5	64	13
6	40	9
7	50	11
8	101	22
9	61	15
10	56	14
Total	619	143

The 143 chromatoid bodies observed are fairly close to the expected number 155.

With the elongation of the spermatid the chromatoid body wanders further and further from the nucleus, and usually lies close to the axial filament (Fig. 12). It migrates eventually to a considerable distance down the tail, where it may be seen, still in its vacuole, forming a swelling at one side of the axial filament in the nearly mature sperms (Fig. 13). When the metamorphosis is complete no bodies are seen in the tails themselves, but scattered among them are numerous deeply staining bodies of the same size in various stages of degeneration. This condition is plainly visible in the living material where the loose granules stain quickly and brilliantly with neutral red. The process is therefore identical with that in *Pentatoma*, except that it is unusual to find any great amount of protoplasm cast off from the sperm tails with the chromatoid bodies.

Staining Reactions of the Chromatoid Body.

As has been noted above the chromatoid body in *Rhomaleum* gives the specific reaction when the living cells are treated with neutral red which has been described for certain granules of similar behavior in *Chorthippus*. Lewis and Robertson have also observed that somatic and apical cells often appear to be crowded with material which gives a similar though more distinct relation. I am able to confirm their observation with regard to the apical cell in *Rhomaleum*. It appears, therefore, that a specific substance which is an ordinary inclusion of the cytoplasm of certain somatic cells and of the apical cell is present in the cytoplasm of the spermatogonial and early growth period cells of some grasshoppers in small quantities, that it increases in amount during the growth period, and may become aggregated into one mass. Like almost any foreign substance in the cytoplasm this mass appears to lie in a vacuole in fixed material. It is finally eliminated from the nearly mature spermatozoa. That a similar condition occurs in the male germ-cells of many other animals is made probable by the fact that similar bodies have been described in an increasing number of forms.

As to what this substance is, one can give no certain answer. In the living cells it remains almost invisible when unstained, and it appears faintly pink after a rather long treatment with neutral red. In the fixed material the body stains densely with hæmatoxylin, safranin and other chromatin stains. When the Flemming triple combination is used the chromatoid body is red throughout, even though the resting nuclei are purple. With the Auerbach stain the body is clearly differentiated from the chromatin, for it is bright red while the nuclei are green. With the Benda alizarin-crystal violet method, even when the material is fixed as directed with the modified Flemming's fluid, the mitochondria seldom appear, and the nuclei and chromatoid body appear bright purple. I have tried this method repeatedly with the germ cells of *Rhomaleum* but have never been able to get the brilliant result shown by Giglio-Tos and Granata (1908) in their paper on *Pamphagus marmoratus*. With the Altmann acid fuchsin method the chromatoid body is clear red as are the mitochondria. The material is therefore unlike either mitochondria or chromatin in chemical constitution, a fact clearly shown by its behavior.

I am glad of an opportunity to express my indebtedness to Professor E. B. Wilson for generous advice in the preparation of this paper as well as for numerous suggestions with regard to technical methods. I am also indebted to Miss Mabel Hedge for the original drawings of Figs. 3 and 4.

SUMMARY.

The mitochondria in *Rhomaleum* are shown to be present in the spermatogonia. Their behavior agrees closely with that described by Lewis and Robertson for *Chorthippus*, except that they remain granular throughout.

There are present in the spermatogonial cells of *Rhomaleum* in addition to the mitochondria certain fine granules which stain in contrast to the mitochondria with neutral red. These granules are carried over into the early spermatocytes where they probably become aggregated into one mass. This mass grows for a short period, passes inert through the two maturation divisions, and into one fourth of the spermatids. From the tails of the developing sperm it is cast off into the end of the follicle, where it degenerates. In addition it has been noted that an idiozome or attraction sphere is present in the early spermatocytes of *Rhomaleum*, and an acrosome sphere in the spermatids.

COLUMBIA UNIVERSITY,
October 5, 1916.

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Explanation of Plate I.

All figures except Nos. 1, 6 and 8 from camera drawings made with Leitz 1.5 mm. oil immersion objective and 8 compensating ocular. Nos. 1, 6 and 8 from living material with 2 mm. objective.

Fig. 1. Resting stage of secondary spermatogonia—living cell stained with janus green showing granular mitochondria.

Fig. 2. First spermatocyte showing chromatin network with cloudy mass of mitochondria and prominent "neutral red granules" in the cytoplasm.

Fig. 3. First spermatocyte beginning to show the "massive bodies"; mitosome, idiozone and "neutral red granules" visible in cytoplasm.

Fig. 4. Similar to the preceding, the idiozome shrunken so as to appear in a vacuole because of imperfect fixation.

Fig. 5. First spermatocyte bouquet stage—diplotene threads polarized toward idiozome, chromatoid body visible.

Fig. 6. Similar to preceding—living cell stained with janus green—mitochondria in two masses surrounding a spherical space in which the idiozome (invisible) probably lies, since the diplotene threads are polarized toward it.

Fig. 7. Diakinesis—drawn from a preparation stained with alizarin and crystal violet—showing large chromatoid body in a vacuole.

Fig. 8. Similar stage in living cell stained with both janus green and neutral red,—mitochondria and chromatoid body visible.

Fig. 9. Telophase of first spermatocyte division—the chromatoid body passing to one pole.

Fig. 10. Late telophase of second spermatocyte division—spermatid nuclei already formed—mitochondria aggregated into the spherical nebenkerns-chromatoid body in one daughter-cell.

Fig. 11. Spermatid showing nebenkern and acrosome sphere.

Fig. 12. Spermatid, tail beginning to elongate, nebenkern divided, chromatoid body passing down close to the axial filament.

Fig. 13. Group of sperm tails, showing chromatoid bodies just before they are cast off.

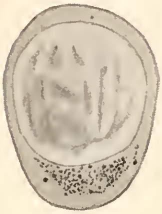


FIG. 1.

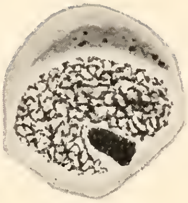
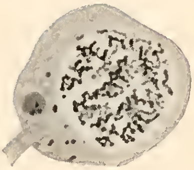


FIG. 2.



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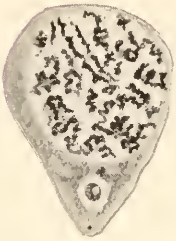

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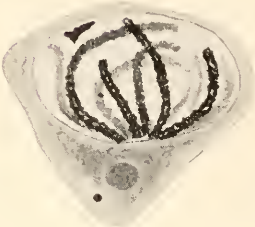
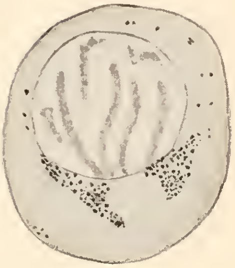


FIG. 5.



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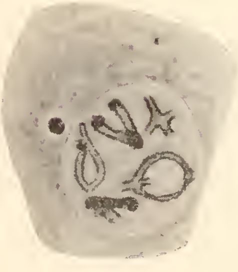

FIG. 7

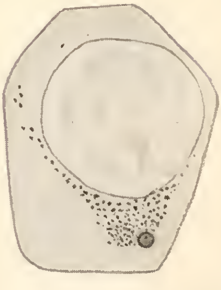

FIG. 8.

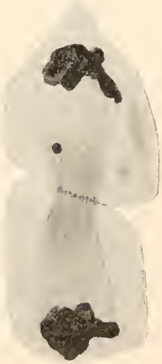

FIG. 9.

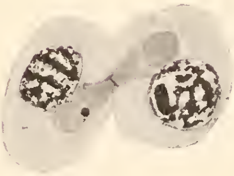

FIG. 10.

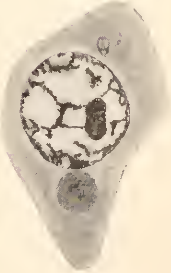

FIG. 11.

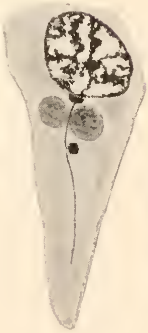

FIG. 12.

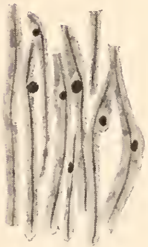

FIG. 13.

HAROLD H. PLOUGH.

ON THE BREEDING HABITS OF DESMOGNATHUS FUSCA.

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That the eggs of *Desmognathus fusca* are deposited under terrestrial conditions, and are brooded by the mother during their development, are facts already well known. In my account of the "Life History of *Desmognathus fusca*" (I. W. Wilder, '13), I made the following observation and suggestion: "The eggs are always found guarded by a female, undoubtedly the mother. She usually so places herself among them as to bring practically all of the eggs in contact with her body, which often extends through the mass of eggs and is frequently bent sharply upon itself as if the better to surround and protect them (Fig. 1).

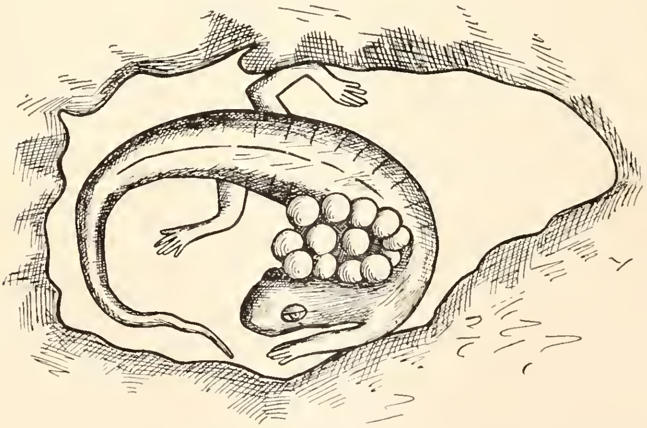

Fig. 1. Female *Desmognathus fusca* with eggs, showing the characteristic brooding position, with the body coiled about the eggs. Drawn from life by H. H. Wilder. From *American Naturalist*, Vol. XXXIII.

When under observation, as in a terrarium, the mother frequently leaves the eggs when disturbed, always retreating through the same exit from the nest. After having been separated from the eggs, however, as may occur in making a transfer from out of doors to the laboratory, the mother goes back to them again, even though the nest and all of its surroundings may have been reconstructed. I have never had the opportunity to further test the sense of ownership of eggs in a mother by exchanging the eggs of two individuals, but the experiment would certainly be an interesting one."

In connection with later experiments upon the mating habits of *Desmognathus fusca*, certain facts have come under my observation which give more definite information concerning this brooding instinct of the mother and her behavior during the brooding period. These facts furnish, in addition, more exact data concerning the length of the incubation period of the species, which, at the time of the publication of my account, was not definitely known.

On July 2, 1915, late in the afternoon, a female *Desmognathus fusca* which had been under observation in the laboratory since May 20, 1915, confined in a terrarium with another gravid female and a male, was found to have deposited a batch of eggs. These had been laid within the previous twenty-four hours, and were in the early segmentation stages when found. The companion male and female were immediately removed from the terrarium and in order to make sure that the eggs were actually those of the female with whom they had been found associated, the companion female was examined and found to be still gravid.

The terrarium in which the eggs were laid was a rectangular glass one, measuring 13 inches long by 8 inches wide by 13 inches deep, and had a two-inch layer of wet sand in the bottom, sloping down at one corner to allow the water to stand in a shallow pool. Upon the sand had been placed wet sphagnum moss, and a stone about 4 inches in diameter was lying upon the sphagnum near the end of the terrarium which was opposite to the pool. It was in a cavity in the sphagnum underneath the stone that the eggs, 22 in number in two clusters, were found with the mother coiled about them in the usual brooding position of the species.

During the subsequent weeks the stone was frequently lifted to examine the nest, in the evening as well as during the daytime, and the mother was always found in this characteristic coiled attitude, although the relative position of the eggs and the mother was slightly changed from day to day. She seemed little disturbed by these brief examinations unless the eggs were actually handled, when she would leave the nest and go away a little distance, always, however, to return to her charge later. On August 23, after an incubation period of 52–53 days, the eggs began to hatch, the process continuing through August 25.

Meanwhile, on July 7, in the late afternoon, another female in a similarly arranged terrarium, was found to have begun to deposit eggs. This female had been captured on the same date as the first (May 15), had shown when captured a similar evidence of the presence of ripe eggs conspicuous through the body wall, and had been similarly confined since May 20 with another gravid female and a male. On July 1 the male had been removed, however, and as soon as the eggs were discovered the other female was removed also. At this time a small cluster of three or four eggs had been deposited, and a single isolated one which was removed at once from the nest for examination, and was at this time unsegmented.

On the following forenoon the egg-laying process had been completed, and the usual two clusters of eggs were found, one numbering six and the other twelve. The cluster of six eggs was appropriated for study of the early cleavage, and it was found that at that time, about twelve o'clock, some of them were in the first cleavage stage, while others showed as yet no suggestion externally of the cleavage process. The one which had been removed on the previous afternoon was at this time in the second cleavage stage.

The eggs of this second batch were deposited in a cavity hollowed out in the moist sphagnum under the stone, as was the case of the first batch of eggs which had been deposited in the other terrarium a week earlier. This second mother, however, took from the beginning an unusual position with reference to the eggs, for instead of lying among them with her body coiled about them, she stood over them with her body in a straight line and slightly elevated so that the eggs were beneath her belly and only slightly in contact with it. Care was always taken in examining the nest not to disturb her or her surroundings beyond lifting the stone carefully from the nest and replacing it with equal care, in exactly the same position. She was invariably found in the same peculiar attitude with relation to the eggs, and always with the head oriented in the same direction.

On August 24, after the eggs of the first batch had begun to hatch, both of the mothers were removed from their respective eggs. The mother of the second batch was killed and preserved. The mother of the first batch, now hatching, was transferred to the terrarium containing the second batch, now motherless, but was placed in the part of the terrarium farthest from the stone under which the eggs were located. Great care was taken to leave the sphagnum surrounding the eggs, and the stone covering them in the same relative position which they had had throughout the incubation period.

On the following day when the terrarium was examined the female was not in sight, but upon lifting the stone, she was found under it brooding the eggs and standing over them in exactly the same unusual position which their own mother had habitually assumed. She remained with them and was always found in the same position, with her body oriented in the same direction, until, on September 2, a week later, all of the eggs had hatched. Upon this date the young were found clinging to the body of their foster mother as she stood with her customary orientation, a number of them surrounding the middle of her body like a rosette, with their long axes parallel to hers but with their heads pointing in the opposite direction. When she was disturbed, a few were somewhat scattered while most of them still clung to her body. After a few days, the adult was found in the little pool at the opposite end of the terrarium, and the young, still in the terrestrial larval stage, were distributed through the moist sphagnum between the nest and the pool.

Of course there is no means for determining whether the actual finding of the eggs by the foster mother was a reaction to the proximity of the eggs themselves, or was a purely accidental occurrence, since *Desmognathus* frequently seeks out positions under stones and other objects lying upon the surface. Obviously, however, in her subsequent week of continual brooding of the eggs, and in her assumption of the very unusual attitude during this period, we have an interesting example of a perfectly automatic response to external conditions. Otherwise her attitude would hardly have shown, as it did, so exact a correspondence to the aberrant one previously assumed by their own mother, but would rather have been the characteristic one which the foster mother had taken in brooding her own eggs. The chief of the conditions controlling this automatic response would seem to have been the presence of the eggs themselves, since, after the foster mother had once reached the pool, she apparently did not return to the stone again but was always found in or near the pool. The peculiar position and orientation of the bodies of the two females while successively brooding this particular batch of eggs is most satisfactorily explained, however, as a response to some unusual conditions in the surroundings of the nest, such as the possible entrance of a little light into the nest from one direction. This explanation receives some corroboration in the fact that a little crevice was noted leading from the surface into the side of the nest toward which the head of the adult was directed. Furthermore, the newly hatched larvæ, which would be expected to be negatively phototropic if they are to succeed in reaching the neighboring water by working their way down through the moist earth and debris, oriented themselves in the direction opposite to that of the mother.

It is moreover possible that we have a further automatic response exhibited by the mother in seeking the water after the young had hatched. This movement toward the water would be of use to the offspring, for they tend to cling to the mother and would thus be eventually guided by her to the water. Under natural outdoor conditions where the nest would be farther away from the water than was possible within the limited dimensions of the terrarium, the time occupied in making this transfer might roughly coincide with the duration of the terrestrial larval stage.

That the terrestrial larval stage is really a definite one is shown by the behavior of the newly hatched larvæ when placed in the water. They are so well developed muscularly that they can not only swim, but can maintain a horizontal position in the water when not swimming, instead of lying on one side as do the newly hatched larvæ of most amphibians. Nevertheless they will not remain in the water, but persistently crawl out and lie, often in a mass together, in the moist debris along the edges. It is not until all external evidence of the yolk mass has disappeared that they will remain in the water.

The period of incubation in both of these broods was approximately eight weeks (53–55 days in the first case, and 56–57 days in the second), a considerably longer time than that previously estimated by me, which was five weeks. The terraria were kept in a cool basement room, where the temperature did not vary much from 21° C. (70° F.), which was probably somewhat above the average temperature to which the eggs would have been subjected under natural conditions along the banks of brooks in shaded ravines. The former estimate of five weeks was based upon observations of a batch of eggs which were deposited and developed under still warmer laboratory conditions. As the measurements and descriptions of an embryo from this batch after 30 days' development (H. H. Wilder, '99) show a considerably larger size and a more advanced stage of development than the 34 day embryos of the batch of eggs here reported, one is justified in the conclusion that at the higher temperature development took place more rapidly. On the other hand, it is conceivable that in nature the period of incubation might easily be prolonged to more than eight weeks by the lower temperature to which the eggs would certainly be subjected in the neighborhood of cold, spring-fed, mountain brooks. Thus the batch of eggs previously described by me as having been found in nature hatching on September 24 (I. W. Wilder, '13), after an unusually cool summer, may even have been deposited as early as the middle of July, the month reported by Reed and Wright ('09) as the month of maximum egg-laying for the species. This longer estimate of the period of incubation under natural conditions would account for the usual absence of the larvæ of this species from the brooks during the summer months, a fact which is reported by my colleague, Mr. E. R. Dunn, in an article now in press.

The female which acted as the foster mother in the case here reported was continued under observation in the laboratory for nearly a year. She was fed abundantly upon *Drosophila*, which, as a "by-product" of genetics experimentation, has proved a valuable laboratory food for adult salamanders. In spite of her well-nourished condition, however, she gave no evidence of the ripening of a new lot of eggs during the following spring and summer, while another female of about the same size (87 mm. in total length), which had not been gravid the previous year, developed under the same care and feeding, large eggs which could be conspicuously seen through the body wall early in the spring. These observations are too limited in number to base any definite conclusions upon them, but they at least suggest that the females of this species do not necessarily produce eggs every year. This hypothesis would also explain the fact that I have found that occasionally females collected in early spring contain no large eggs. The number of offspring in this species is in any case very limited, as shown by the small number of eggs in a batch. These average about 20 in the cases which have come under my observation in western Massachusetts, while the largest number of ripe eggs which I have counted in the ovaries of a single individual is only 28, and the number in a batch may run as low as 14. If in addition to this characteristically small number of eggs produced at a time, the females sometimes fail to produce eggs every year, there would be a still further limitation in the number of offspring. Such a reduction has been made possible only by the high percentage of success in the development of the few eggs which are produced. One of the conditions contributing largely to this success is the large amount of yolk present in the egg, which makes possible the attainment of a considerable size and maturity at the time of hatching in consequence of which the larvæ are better able to take care of themselves. A second condition insuring the success of the offspring is seen in the internal fertilization which insures the impregnation and development of every egg which is deposited. In fact, my experiments have shown that gravid females which are isolated from the males early in the spring and thus fail to become fertilized, do not deposit their eggs at all, and that by the middle of August the eggs are already undergoing rapid resorption.

This fact shows an extreme illustration of the conservation of material in this species, and is quite in line with the conservation shown in the reduction of the number of eggs. It shows a decided advance in comparison with the habits of certain other amphibians such as *Cryptobranchus alleghenicnsis*, for example, which is prodigal in its egg production, but often uses its own eggs as food (Smith, '07). Finally, it is certain that a most potent contributory factor to the high percentage of success in the development of the offspring of *Desmognathus fusca* is found in the extraordinary constancy and devotion of the mother to her offspring during the incubation period, a devotion no less effective because it is an automatic response.

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THE SYMMETRY OF GRAFTED EGGS IN RELATION TO GIANT LARVÆ FORMATION IN ARBACIA PUNCTULATA.

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TABLE OF CONTENTS.

	PAGE
Introduction	21
Single Larvæ, Axes of Two Grafted Members Unknown	22
Single Larvæ, Axes of Two Grafted Members Known	23
Single Larvæ, Absorption of One Member	24
Change of Axes During Development	27
Summary and Conclusions	31
Bibliography	32

Introduction.

Two or more eggs have been experimentally grafted together by several investigators (Driesch, Goldfarb and de Hahn, 2, 3, 4, 5, 6, 7 and 8), who have also described some of the types of larvæ resulting from such grafts. One of these types, the Riesenlarva, first produced and described by Driesch, has aroused considerable interest particularly with respect to its origin. This larva is distinguished from other fused larvæ, in that it is normal in structure, single, and identical with control larvæ, except for size. According to Boveri (1) and de Hahn (5) such perfect fusion into a single larva can occur only when the axes of the fusing eggs are so placed that they bear the same relation to each other as the blastomeres of the two cell stage of an egg; *i. e.*, the axes and the planes of the two grafted eggs must be parallel and symmetrical.

In studying separate clusters of grafted eggs of the sea-urchin *Arbacia punctulata*,[1] and in *following the development of each cluster through its pluteus larva*, numerous facts were disclosed that did not accord with Boveri's hypothesis of the genesis of the Riesenlarva. A concise statement of these facts is given below, and for the sake of brevity only a few stages in the development of each cluster will be shown, all drawn to the same scale except where specifically mentioned to the contrary, and all drawn from the living specimens with the camera lucida.

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[1] For technique of grafting, see Goldfarb 6 and 7.

Single Larvæ, Axes of the Two Grafted Members Unknown.

Under this heading are included those clusters which developed into single perfect larvæ, and in which the axes at the beginning were either unknown, or could not be definitely ascertained.

Fig. 1 is a foreshortened view of a pair of blastulæ, partially fused together. The diameter of each component when compared with the controls clearly shows that these are two blastulæ and not two half blastulæ. The axis of each member could not be definitely ascertained until the blastopore, or better still until the gut, is differentiated. Fourteen hours after the stage shown in Fig. 1 the pair was transformed into a "single" gastrula, with a "single" invagination shown in Fig. 2. This gastrula developed into a "single" typical larva (Fig. 3), which was essentially unchanged during the next four days. This larva contained one skeleton and one gut, and if its history had been unknown might readily be mistaken for a true single larva derived from a single egg.

Fig. 4 is another double blastula which developed into a "single" large gastrula, and then into a young pluteus containing a single pair of skeletal spicules, and a single gut (Fig. 5). Three days after fertilization, it developed into what appears to be a single perfect larva, shown in end view (Fig. 6). During the next four days there was no essential change except the elongation of the anal arms seen in Fig. 7 from a different view.

Four stages are shown in the development of the next specimen. Fig. 8 shows the giant blastula somewhat foreshortened. Fig. 9 drawn seven hours later, shows the giant gastrula with its single gut a little to one side; fourteen hours later the gut is more sharply to one side (Fig. 10). This gastrula developed into a "single" short and somewhat atypic pluteus, containing a single gut, still a little to one side, and a single asymmetrical skeleton with one side distinctly enlarged (Fig. 11).

In the preceding as well as in many other examples, the grafted pair of eggs developed first into a double blastula and later into a single gastrula. All subsequent development was single. In all of these instances it was impossible to determine the axis of the second member, for only one blastopore and one gut was formed. It is possible that the eggs, in these instances, were by chance grafted to each other in the same relative positions as the blastomeres of an egg, and that as a result of this position the grafted pair developed into a single organism as required by Boveri and de Hahn. That this possibility is very remote will be shown in the following sections. Let me first draw attention to such instances in which the axes of both members are known.

Single Larvæ, Axes of the Two Grafted Members Known.

In this section the axis of each member was definitely established by the definite formation of a gut in each member.

Fig. 12 is a foreshortened view of a nearly equal pair of fused gastrulæ whose embryonic guts and therefore whose axes are about 135 degrees apart. This double gastrula developed into a "single" larva (Fig. 13) which grew into a larva decidedly larger than the controls (Fig. 14). This larva contains certain accessory parts which are not uncommon in fused larvæ, at least in certain stages of their development. There is, for example, a small accessory oral rod at X, which structure is sometimes found in control larvæ. There is also an accessory fold of the gut, making four in place of three characteristic divisions of the gut. This condition is very unusual if not entirely absent in true single larvæ. This example is but one of a series which differ only in minor details and which show that "*single*" *giant larvæ may be formed even when the axes of the two grafted members* are not parallel and not symmetrical. How the two guts are moulded into one, and how the relative position of the axes is changed will be considered later.

Whether the relative position of the axes is or is not permanent, one would expect from Boveri's and de Hahn's hypothesis, that two members, which were clearly not symmetrical, in the specimen shown in Figs. 12 to 14, would not develop into a single larva, which is contrary to our observations.

Another double gastrula is shown in Fig. 15, in which the two guts are unequal and in which the *axes are bent at an angle approximately 45 degrees. This double gastrula developed 24 hours later into a "single" larva*, and normal except for a slight swelling of the body wall in the aboral region on one side. Fifty-four hours later it developed into a very large larva (Fig. 16), whose body, skeleton and digestive tract are "single" and normal except for the hypertrophied bar in the aboral region marked X.

I have records of ten other similar pairs of grafted gastrulæ in which the relative sizes of the guts and the axial angle varied, yet in spite of this asymmetry, all of them developed into "single" larvæ like those just described. Two other pairs are described in the next section (see Figs. 26 and 34).

In these instances at least, the particular angle formed by the axes is not correlated with the formation of Riesenlarva, and in all of them *in spite of marked axial asymmetry, the two members fused into perfect or nearly perfect "single" larvæ, and Riesenlarvæ.*

Single Larvæ by Absorption of One Member.

In all the clusters under consideration *there was no separation of the two members.* Separation frequently occurred especially in agglutinated pairs, but such specimens were of no significance in these studies and are omitted from consideration. All of the "single" larvæ described in this paper had their genesis in a pair of more or less completely and permanently fused blastulæ or gastrulæ.

Many grafting experiments have shown that a resorption of parts often takes place, and it was conceivable that in these experiments resorption or disintegration of one of the members might also have taken place. Before any conclusion can be drawn it must be definitely shown whether resorption or disintegration occurred and the nature and degree of resorption. The following examples will throw some light on this phase of the problem.

Fig. 17 is clearly a fused pair of blastulæ approximately equal in size. During the next twenty-four hours the two developed very unequally, one into a gastrula and then into a young pluteus, while the other ceased developing and then decreased in size

(Fig. 18). During the next two days the non-developing member continually decreased in size, and finally disappeared altogether; its mate progressively differentiated and grew into the "single" larva (Fig. 19) which is larger than control larvæ. This example is typical of a fairly large group in which one member is slowly but completely absorbed and in some instances disintegrated.

A stage between that shown in Figs. 17 and 18 is represented in the next example. This grafted pair consists of a blastula and a somewhat larger gastrula. A free-hand sketch is shown in Fig. 20. During the next twenty-four hours the blastula diminished in size and gradually disappeared, while the gastrula developed into a normal young and single larva (Fig. 21). During the next five days this larva enlarged considerably, accessory skeletal bars and accessory gut appeared, as seen in foreshortened view in Fig. 22. During the succeeding four days besides the expected reduction in size of the larva, a number of accessory skeletal bars also disappeared from both anal and arm rods, making the larva resemble even more closely single control larvæ.

In the next example a blastula is grafted to a gastrula (Fig. 23). The blastula gradually diminished in size and disappeared without further differentiation, while the gastrula grew and differentiated into a "single" larva. This larva has a single gut bent a little to one side, with a single skeleton plus an irregular accessory rod connected to the basal and aboral rods of the skeleton (Fig. 24). During the next four days, while the larva continued to grow, two accessory parts of the gut appeared making a five part digestive tract in place of a tri-partite gut as in normal animals. The hind and mid guts are in duplicate. The accessory bar is also enlarged and is much fenestrated. In this example as well as in the preceding, one of the grafted members clearly disappeared BUT DURING AND AFTER THE DISAPPEARANCE OF THIS MEMBER ACCESSORY PARTS APPEARED IN THE SKELETON AND IN THE GUT OF THE DOMINANT MEMBER.

Such accessory parts appeared in all or nearly all cases after one member disappeared, and was frequently associated with a general enlargement of the body. Accessory parts are not rare among controls, but they do not occur as frequently nor are they of the character, nor are there so many accessory parts in a single organism as in these fused larvæ. These and other facts (see Goldfarb 7 and 8) appear to me to indicate that one of the members is not disintegrated, nor merely absorbed. It appears to me that the cells of the smaller or weaker or more slowly differentiating member are translocated, into the dominant member, where the translocated cells are regrouped into additional gut, skeleton or body wall, and in cases of incomplete regulation, into accessory parts.

Absorption and translocation of cells of one member may take place even after both members have fully differentiated their guts, and the axes of both members was definitely fixed. For example, two grafted eggs developed into a double fused gastrula, one somewhat larger than the other and with axes about 90 degrees apart. A free-hand sketch is shown in Fig. 26. During the next twenty-four hours the smaller gastrula was gradually absorbed, while the other continued its normal development. The resulting larva which is shown in Fig. 27 consists of one body, one skeleton, all normal except for the small accessory bars at the aboral swollen end of the body. During the next two days the larva became decidedly larger, the swelling at the aboral end smaller and concomitant with these changes, two accessory parts of the gut appeared (Fig. 28). During the next three days while the body gradually diminished towards the normal, THE ACCESSORY GUTS LIKEWISE DISAPPEARED AS WELL AS THE ACCESSORY SKELETAL PARTS, *transforming the larva into a completely normal one* (Fig. 29), indistinguishable from control larvæ.

In this instance the two fused gastrulæ, although their axes were 90 degrees apart, gave rise to a "single" larva, as did the paired gastrula of the preceding section. *If the intermediate steps in the development had not been observed, one might have concluded either that this larva was derived from a single egg, or from a symmetrical pair of eggs as required by the Boveri-de Hahn hypothesis.* But it is evident that *the process was quite different, that there was first a gradual and definite absorption of one member, a translocation or migration of parts, an increase in the volume and number of parts of the dominant member, and finally a loss of materials and parts.*

These successive changes do not always give rise to normal larvæ. Atypic or incomplete larvæ may be formed. I have many records of the transformation of pairs of grafted gastrulæ into atypic enlarged or incomplete single larvæ, which will be described elsewhere. They are the resultant, from the present evidence at least, of a disturbance in the translocation and rebuilding of cells that make up the skeleton, rather than a resultant of asymmetrical positions of the fused members.

Change of Axes During Development.

In studying the development of fused gastrulæ, it became evident that in many instances the relative position of the axes was definitely altered, so that the angle formed by the two guts and therefore the symmetry of the two fused members, was profoundly altered during development. For example, in Fig. 30, two nearly equal gastrulæ were fused in such a manner that, though their blastopores are nearly united, their guts diverged about 60 degrees from each other. During the next six hours, besides increasing in size, and besides an unequal growth of the two guts, the relative position of the *axes had shifted from 60 to nearly 80 degrees* (Fig. 31). This double gastrula gave rise, by the process of absorption, to a "single" larva, with a somewhat incomplete skeleton and single gut (Fig. 32).

Fig. 33 is a drawing of two fused gastrulæ twenty-four hours after fertilization. One member has its gut fully formed, the other member has just begun to differentiate it. Their axes are about 90 degrees apart. During the next seven hours two changes took place, firstly, the smaller grew relatively faster and became as large as its neighbor, secondly, THE RELATIVE POSITION OF THE TWO GUTS HAS SHIFTED FROM ABOUT 90 DEGREES TO 130 DEGREES (Fig. 34).

In the next specimen the two equal gastrulæ are fused in such a manner that their axes were about 140 degrees apart. Fig. 35 is a free-hand drawing of this pair. During the next two days the gastrulæ developed very slowly and unequally into a nearly full-grown pluteus, and a gastrula just beginning to differentiate its triradiate spicules. *Their axes had in the meanwhile shifted from about 140 degrees to 180 degrees* (Fig. 36).

The next example consists of a pair of somewhat unequal gastrulæ fused so that their axes are about 135 degrees apart. Fig. 37 is a free-hand drawing of this pair. These gastrulæ developed into two fused larvæ *whose axes had rotated from 135 to about 170 degrees* (Fig. 38). In this pair of larvæ it is interesting to note that one half of one member has been completely suppressed.

In the following pair of gastrulæ the AXES ROTATED IN THE OPPOSITE DIRECTION, AND BECAME SECONDARILY PARALLEL, having shifted from about 70 degrees to 0 degrees. The gastrulæ were nearly equal in size and their axes diverged about 70 degrees (Fig. 39). During the next twenty-four hours no material change occurred either in size or differentiation of parts, BUT THE GUTS HAD BECOME PARALLEL (Fig. 40), and ALTHOUGH THESE GASTRULÆ WERE NOW PARALLEL THEY DID NOT FUSE INTO A "SINGLE" LARVA as required by Boveri's hypothesis. It might be urged that while the axes were parallel the two gastrulæ were not necessarily blastomerically symmetrical. Inasmuch as I failed to observe the skeletal spicules which would have established the planes of each of the members, I can offer no opinion, in this example.

In another example a completely differentiated gastrula was fused to one just beginning to differentiate its gut (Fig. 41). This pair is like the one shown in Figs. 33 and 34. Two days later the pair developed into two fused larvæ in which the axes had changed from about 100 degrees to 40 degrees (Fig. 42). This pair is also interesting because one individual developed into a perfect larva, while the other developed into a perfect half larva, the two having a common foregut.

Still more interesting is the pair of gastrulæ shown in foreshortened view in Fig. 43. The axes are about 180 degrees apart, nevertheless during the next seven hours THE AXES HAD SHIFTED FROM ABOUT 180 DEGREES TO 0 DEGREES (Fig. 44). Unlike the pair shown in Fig. 40, these *gastrulæ tended to approach a symmetrical position, yet in spite of this, developed into two larvæ*, one of which was quite irregular (Fig. 45). Much to my surprise the axes continued to rotate during late differentiation, but the skeleton and the gut rotated unequally, for the skeleton shifted from 0 to about 80 degrees and the gut only to about 45 degrees.

In the next example also, the axes tended to approach a parallel position, but the blastopores were 180 degrees apart. The two gastrulæ had their axes originally about 60 degrees apart (Fig. 46). The next day with the development of both retarded, their axes had shifted to about 180 degrees apart and their size and degree of differentiation were more unequal (Fig. 47). This pair developed into a "single" somewhat irregular larva.

Fig. 48 is another instance of axial rotation towards the zero point. This figure is a free-hand drawing of two nearly equal gastrulæ whose axes were 180 degrees apart. The next day the axial angle had shifted about 75 degrees. This angle was maintained throughout subsequent development. The resulting double larva is shown in Fig. 49.

It will be evident even from these few examples that the relative position of the axes may be, and had in fact been shifted during early development of the gastrulæ; that the shifting took place towards or away from blastomeric symmetry of the axes, and that the range of movement was surprisingly large, from about 20 to 180 degrees on the one hand and from 180 to 0 degrees on the other.

It is conceivable that with such shifting of the axes, an original asymmetrical pair may subsequently become symmetrical, and we should then expect from Boveri's hypothesis that development would then be single and Riesenlarva would result. But such Riesenlarva may or may not come out of such symmetrical pairs. That Riesenlarva may be formed in this way cannot be denied. But that there is any necessary relation between symmetrical position of the axes and Riesenlarva formation, is extremely doubtful in view of the examples mentioned above.

On the other hand, if symmetry is not established either by rotation of the axes or by the original position of the pair, we should not anticipate the formation of single larvæ. But as I have shown, such results do occur in at least the four cases described in Figs. 30 to 32, 26 to 29, 15 to 16, 12 to 14.

There is of course the possibility that while the axes may be parallel and symmetrical yet the planes through these axes may not have been parallel, and as de Hahn points out correctly, in the absence of symmetry of the planes, the two members are not really blastomerically symmetrical.

If on the other hand there is but a single example of the development of a Riesenlarva from asymmetrical members, it suffices to overthrow Boveri's theory. And it is unnecessary to demonstrate that two grafted members must be symmetrical. But in four examples at least the axes and planes were clearly asymmetrical, yet they developed into Riesenlarva. At least five others were cited in which the axis of only one member was known, but which also developed into single larvæ. And it is highly improbable that in all these instances the axis of the second member should by a rare combination of circumstances have been blastomerically symmetrical with the known member.

From these results it must be evident that not all Riesenlarva are formed by the fusion of blastomerically symmetrical eggs. While Riesenlarva may be formed in this manner, my observations lead me to conclude that the more common method is by a resultant of many and complicated processes which may briefly be summarized as follows: (1) One member develops normally and completely, the other is arrested in its development, rarely proceeding beyond the gastrula stage. (2) The arrested member is subsequently absorbed, very gradually, and some or all of the cells translocated. (3) The translocated mesenchyme cells form additional skeletal material either making a giant skeleton possible or forming accessory skeletal bars or rods. A translocation of endoderm cells also, either helps in the making of a giant gut, or in forming accessory parts to the gut. (4) With reduction in size, consequent upon starvation of the plutei in the later stages, some or all of these accessory parts tend to disappear. The result of all these changes is frequently a single typical Riesenlarva or atypic double larvæ. In other words the factors making for complete regulation are either not associated with original symmetry of the grafted pair, or with any subsequent symmetry, or they play a very minor role.

Of far greater importance is: (1) the stage in development when fusion takes place; (2) and inequality in the fusing pairs. The earlier the fusion the more complete is it, and the greater the tendency to perfect fusion and Riesenlarva formation. This was in part observed by both Driesch and de Hahn, and Goldfarb. At least any difference in the two fusing members either in size or rate of development, or possibly in vigor or metabolism, is almost certain to be followed by the train of events just enumerated.

It might still be urged that Riesenlarva are formed only when the eggs are blastomerically symmetrical at the moment of their agglutination or fusion; that if the fused pair become symmetrical at any later time, as was shown to be possible, such symmetry was of no avail. But since there is no definite means of determining the polarity of the sea urchin eggs at the time they are definitely fused together, no positive data can be given for or against this possibility, with this material.

Several instances were shown in which asymmetry from the earliest observable moment was followed by single larva formation, and symmetrical pairs did or did not result in single larvæ.

And finally a word may be said about the polarity of the grafted members. That there is a shifting of the axes, involving the body wall, the gut and the skeleton, has already been pointed out. That mesenchymal and endodermal cells may be shifted was shown by Driesch and Goldfarb, but I cannot translate these changes as effecting the polarity of either member.

Following the absorption of the connecting ectodermal wall between the body cavities of the two members, the pair tend increasingly to reach a state of form equilibrium. This involves the migration of the mesenchyme and endodermal cells, with the consequent change in position and shape of the several structures. There is a mechanical shifting of some cells but not a change of polarity of the cells or of the embryo.

Summary and Conclusions.

1. The development of each pair of fused blastulæ of the sea-urchin *Arbacia punctulata*, was studied separately, and some of these pairs gave rise to single larvæ (Riesenlarvæ).

2. Such larvæ are not consequent upon blastomeric symmetry of the two fusing members, as urged by Boveri and de Hahn.

(*a*) The axis of each member is first definitely known at the gastrula stage.

(*b*) One or both members may differentiate their guts and thereby establish the axes.

(*c*) Single giant larvæ may develop when only one gut was differentiated and only one axis formed in the double gastrula.

(*d*) Single giant larvæ may develop when both guts are differentiated and both axes are known.

(*e*) When these axes are clearly and definitely NOT PARALLEL AND NOT SYMMETRICAL the pair nevertheless gave rise to single giant larvæ.

(*f*) Vice versa, when the axes were parallel single giant larvæ were not developed, but various types of double fused larvæ.

3. It was ascertained that the axes of the two fusing members are frequently shifted and rotated towards or away from blastomeric symmetry and with a remarkably large range of movement; that this shifting towards symmetry had no effect upon the formation of single larvæ; and finally that this rotation of the axes did not affect the polarity of the fusing members.

4. The history of the changes in fused members showed that Riesenlarva may be formed when two fusing eggs are not blastomerically symmetrical; that a complex series of changes take place independent of such symmetry; that these changes are associated with an inequality in the two grafted members, an inequality in size or rate of differentiation, or vigor; that there is a definite tendency for the smaller or slower or less vigorous member to be suppressed in its development, that part or the whole of this member may be absorbed, that a translocation of cells may take place and may develop accessory parts or form enlarged organs in the dominant member. That with starvation there occurs a partial or complete absorption of accessory parts, and reduction in size of the larva. The result of these complicated series of regulatory changes is sometimes the formation of a single giant larva, or a single normal size larva, which, if its history were not known, could not be distinguished from control larvæ.

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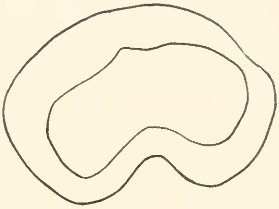
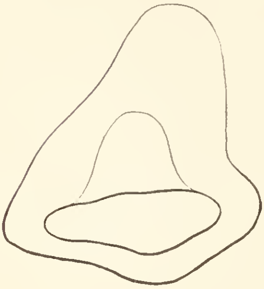


FIG. 1.



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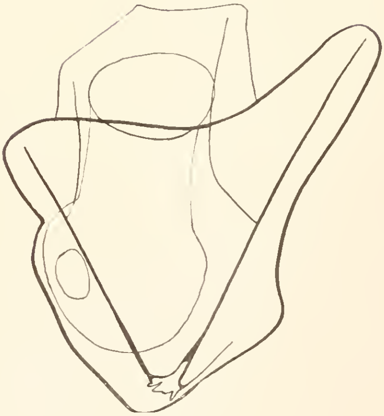

FIG. 3.

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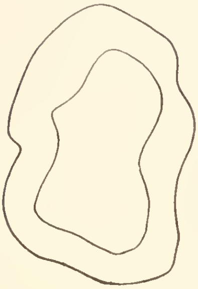

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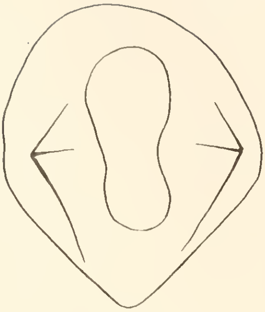

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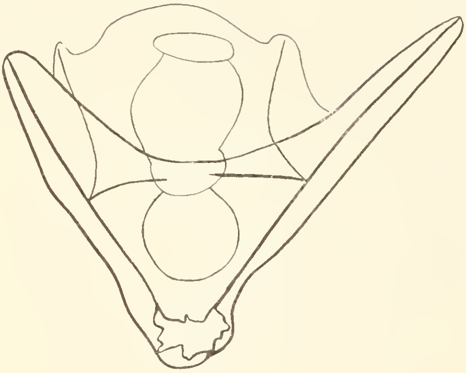

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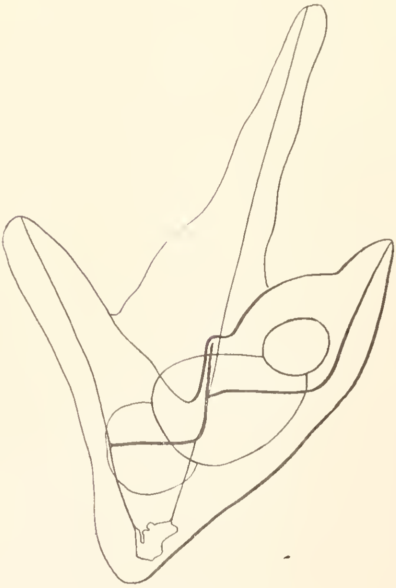

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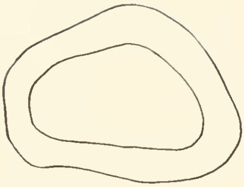

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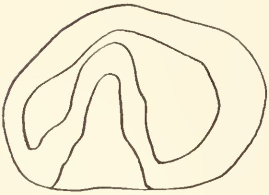

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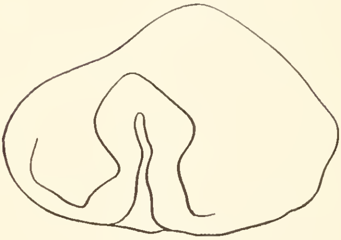

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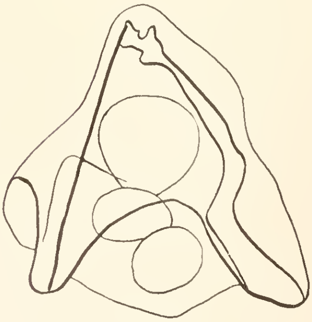

FIG. 11.

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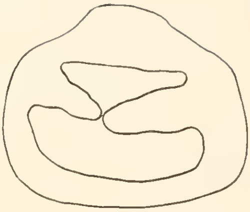

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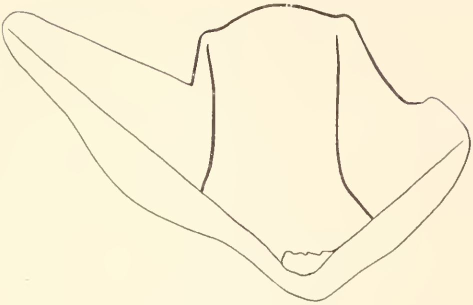

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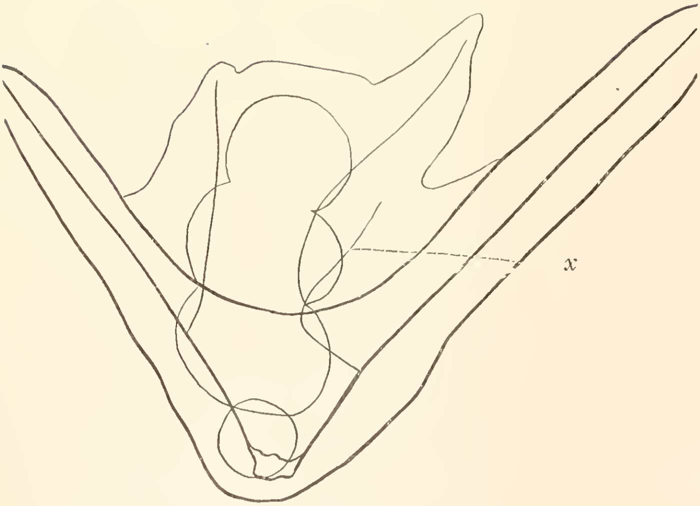

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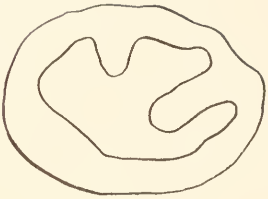

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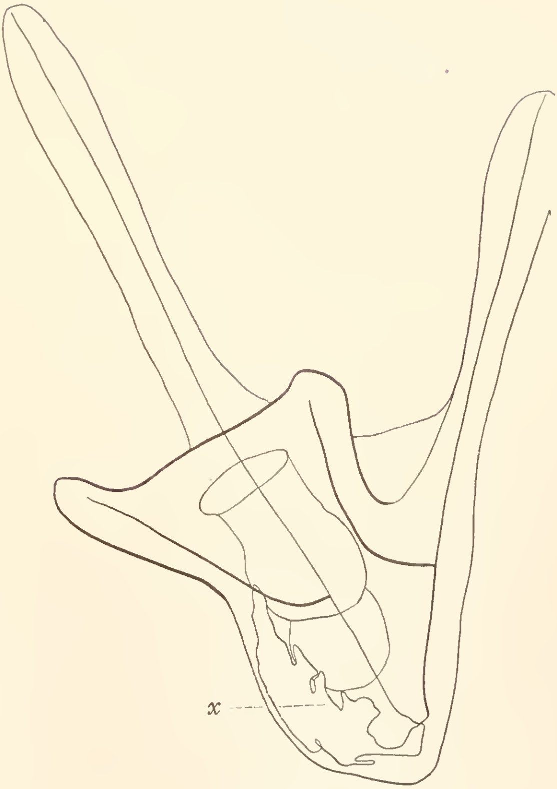

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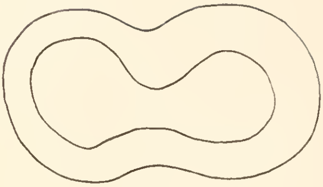

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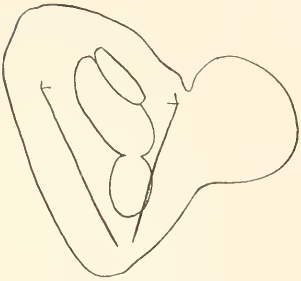

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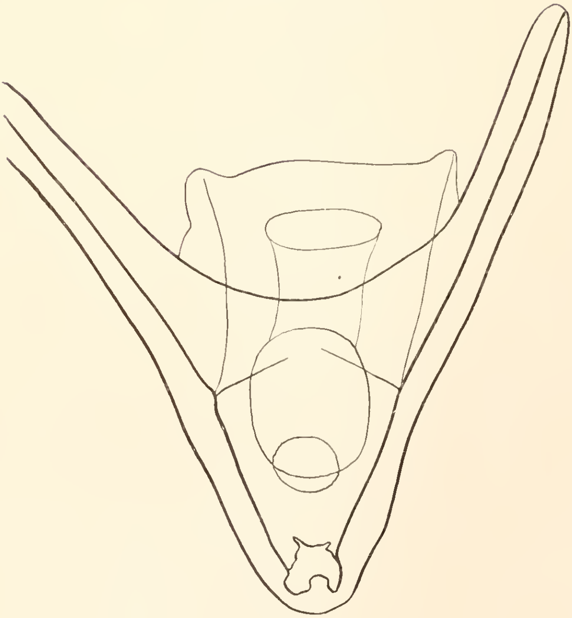

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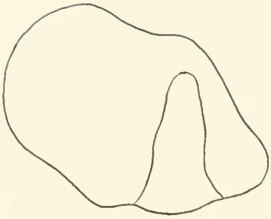

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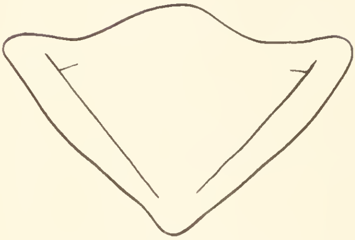

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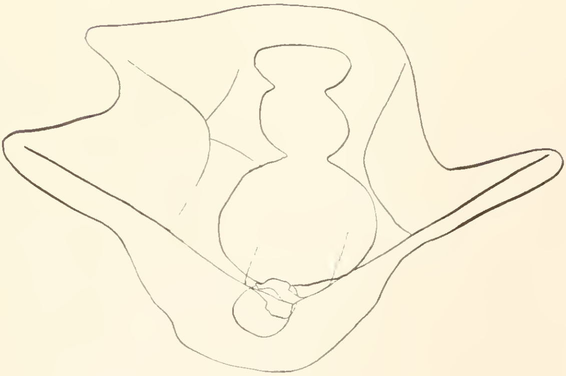

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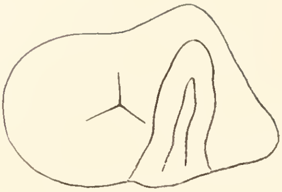

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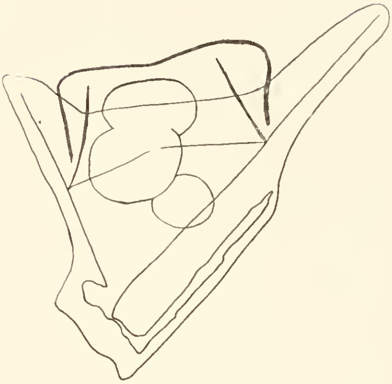

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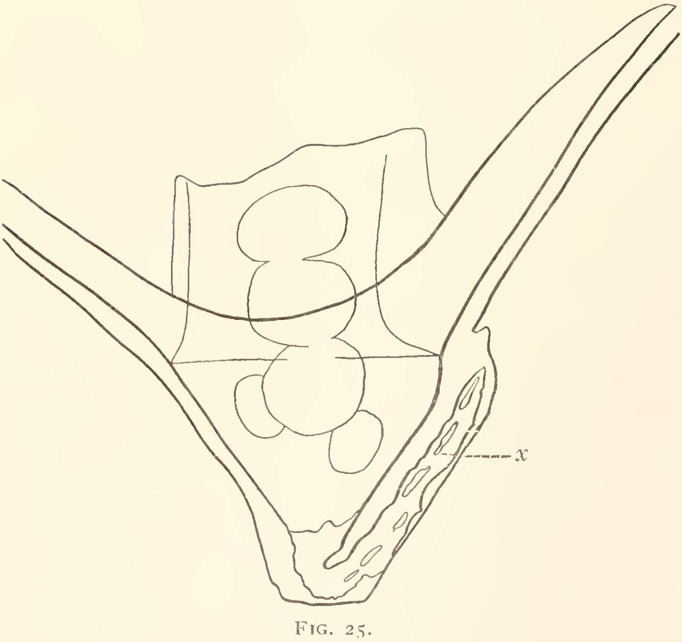

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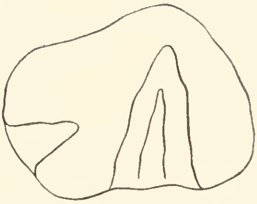

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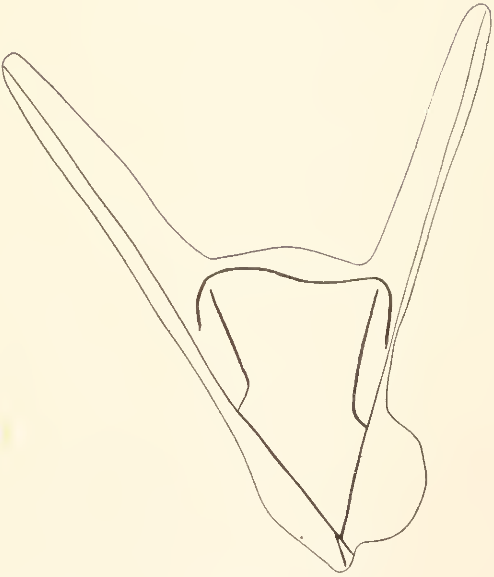

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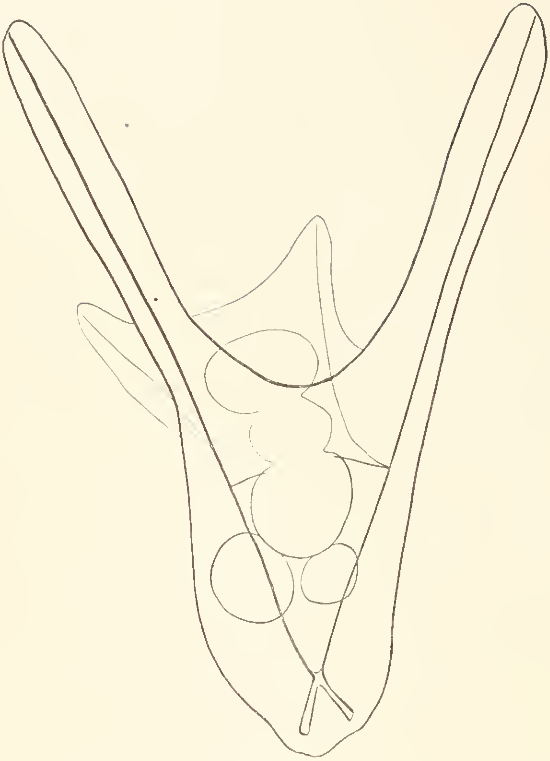

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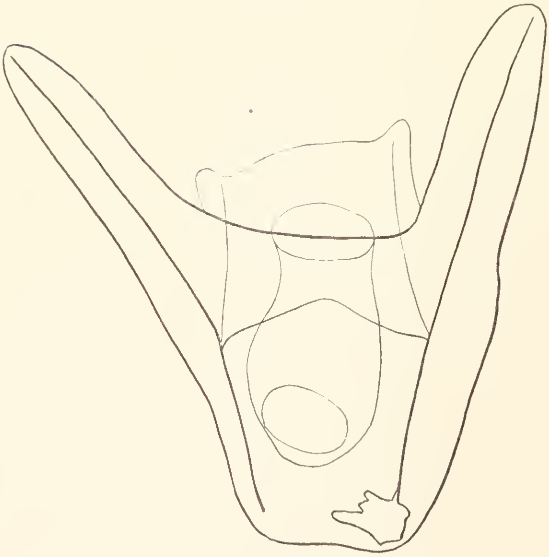

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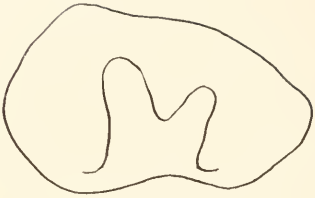

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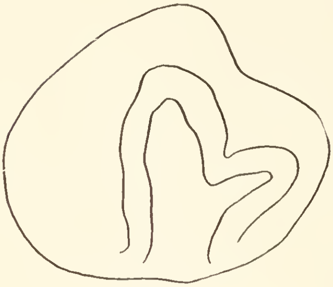

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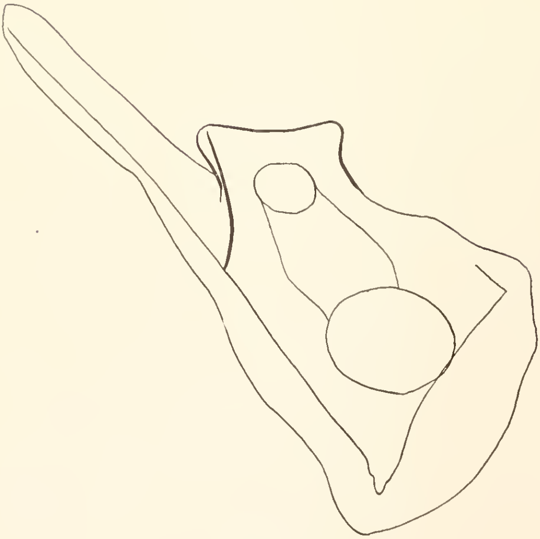

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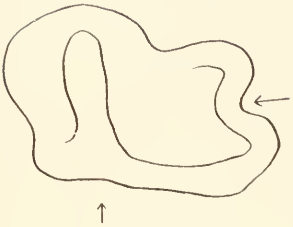

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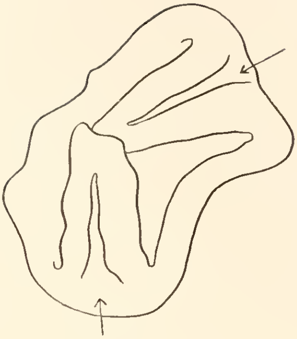

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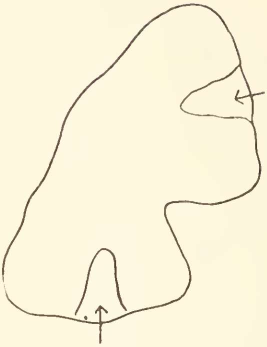

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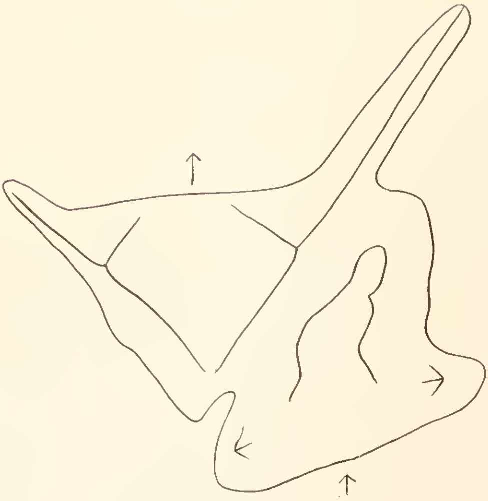

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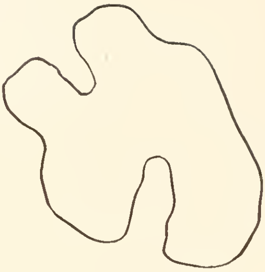

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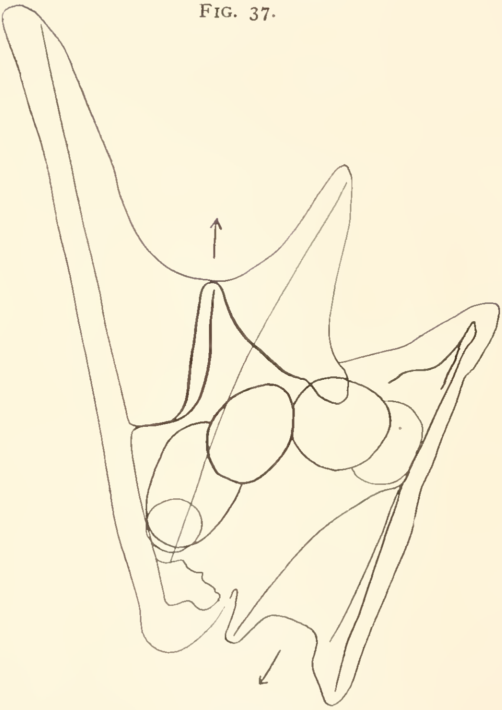

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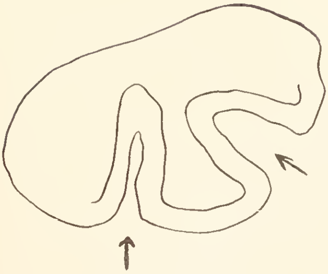

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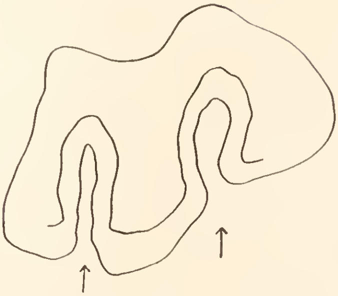

FIG. 40.

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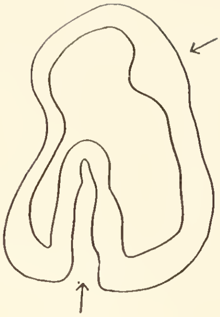

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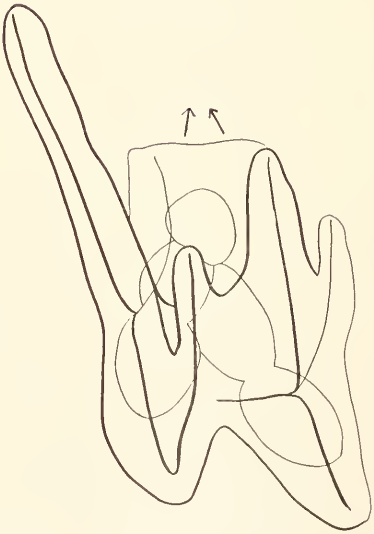

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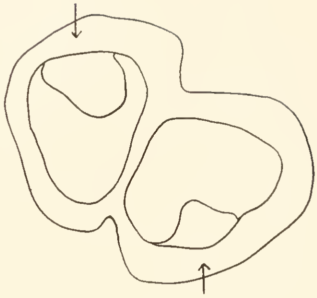

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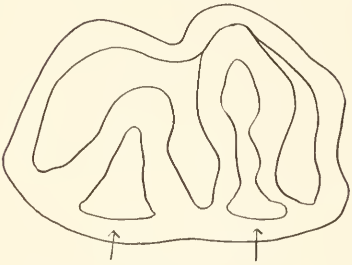

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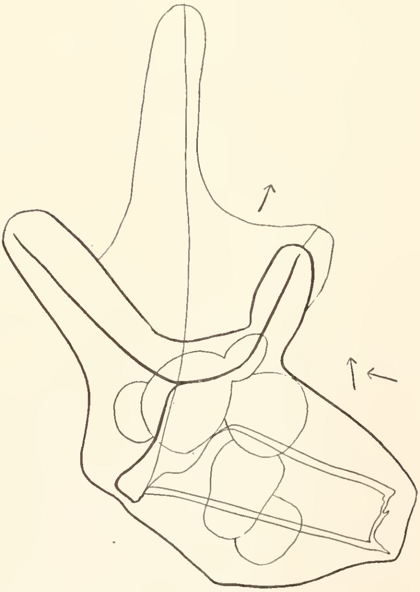

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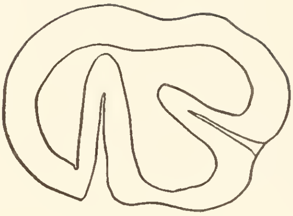

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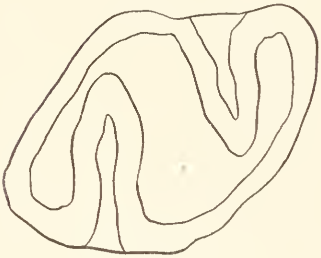

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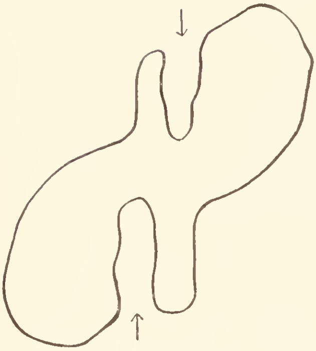

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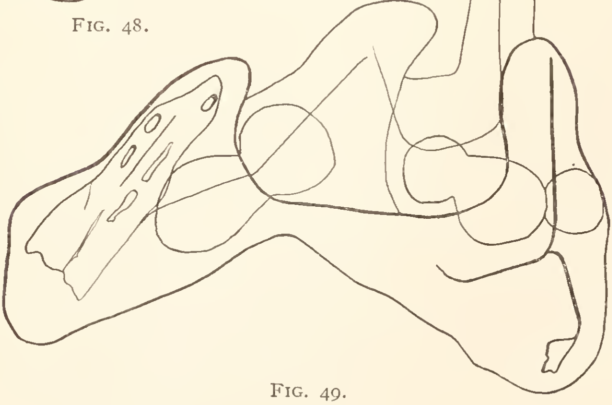

FIG. 49.

A. J GOLDFARB.

ON A CASE OF FACULTATIVE PARTHENOGENESIS IN THE GYPSY-MOTH LYMANTRIA DISPAR L. WITH A DISCUSSION OF THE RELATION OF PARTHENOGENESIS TO SEX.

RICHARD GOLDSCHMIDT,

KAISER-WILHELM INSTITUT FÜR BIOLOGIE, BERLIN.

In the discussion about the relation of parthenogenesis and sex, revived after the discovery of the sex-chromosomes, cases of facultative parthenogenesis in normally bisexual animals play a rather doubtful part. As the great majority of such reports belongs to the older and oldest literature, modern writers appear sceptical in regard to their reliability. Therefore any new case recorded under reliable conditions must command the attention of biologists.

The majority of the reports about facultative parthenogenesis relate to the order Lepidoptera. In most cases only the hatching of caterpillars from unfertilized eggs has been observed, which is of no interest for us here. There are comparatively few cases where adults have been raised and their sex stated. One set of observations relates to the silk-moth. This case can be regarded as settled. There can be no doubt to-day that there are races of the silk-moth which exhibit regularly the phenomenon of facultative parthenogenesis and that the normal number of both sexes is produced from parthenogenetic eggs (for example, see Hartmann, '12). Another set of facts relates to the Psychidæ, where parthenogenesis is a normal phenomenon, resulting in female offspring. A third series of reports deals with occasional cases of parthenogenesis in the gypsy-moth, *Lymantria dispar* L. In 1870, H. Weijhenberg, Jr., reported that he had succeeded in breeding 27 adults, 14 ♀ and 13 ♂, from 60 virgin females of this moth. He also obtained a second parthenogenetic brood, but he does not give details (see Dohrn, '71). A second report comes from G. Platner ('88). He claims to have obtained parthenogenetic eggs regularly by keeping the females isolated for a prolonged period. He states, furthermore, that the maturation-divisions in these eggs are normal. However, a full report of this work never appeared. Since that time many investigators have tried to get parthenogenetic offspring from the gypsy-moth, always in vain. I know that many of those who worked experimentally with the gypsy-moth have tried it again and again, without any success. We and some of our students have used most of our odd material in females for this purpose during the last seven years. The complete failure in every case made us, as well as other observers, believe that the old reports must be based on experimental errors. Nevertheless they seem to be true, for I finally, in 1915, succeeded in getting a parthenogenetic egg-batch.

In this case the conditions of the experiment excluded the possibility of error. I had isolated, for a selection experiment, 4 caterpillars, which were kept in a fruit-jar with a tightly screwed tin cover. After pupation three pupæ died from polyhedra disease. The fourth, a female, hatched. As the experiment was spoiled I left this individual in the closed jar, intending to kill it later. When I opened the jar after a few days the female was busy laying a regular normal egg-sponge, which is normally never done by unimpregnated females. From this parthenogenetic egg-batch, containing certainly over 200 eggs, 22 caterpillars hatched in the spring 1916 and were bred with special care. Three died in early stages and the sex could not be ascertained. Three died before pupation. They were females. Three were killed between the third and fourth moult for cytological study, and proved to be one female and two males. The remaining thirteen hatched and were three females and ten males, the total thus being seven females, twelve males and three unknown. The three females were tried again for parthenogenesis and one of them after ten days finally laid a normal-looking egg-batch. There can, therefore, be no more doubt that facultative parthenogenesis occasionally occurs in the gypsy-moth and results in the production of both sexes. We do not, however, know whether favorable external conditions or some hereditary or not hereditary quality is responsible for the occurrence. The parthenogenetic mother was, in our case, an

F_1-hybrid between a European and a northern Japanese race of gypsy. But neither the parental races nor the other F_1 and F_2 individuals produced parthenogenetic eggs, although ample opportunity was given to many isolated females. This fact neither excludes nor favors the possibility of parthenogenetic strains or mutations.

In order to give an interpretation of these facts it is very important to know the behavior of the chromosomes of these parthenogenetic eggs. A study of the maturation divisions was, of course, impossible. But we were able to ascertain that ovogonia, as well as spermatogonia, of the parthenogenetic caterpillars contained the normal (diploid) number of chromosomes. A visible difference between the chromosome-sets of the two sexes does not, however, exist in the gypsy-moth. The literature on parthenogenesis contains, so far as we are aware, only two statements which relate to our case. One is Platner's already quoted paper, where he states that the reduction-divisions in parthenogenetic dispar-eggs are normal. However, he does not mention the chromosome numbers and we do not know whether the eggs studied by him would have developed. Henking ('92) studied the reduction-divisions of parthenogenetic silk-worm eggs and found a normal reduction-division. But his eggs never developed embryos; therefore his results are of no value for us.

For a real understanding of the relation of parthenogenesis to sex it is very important to know how the diploid number in other parthenogenetic animals is formed. If we compare our case with others where the cytology of parthenogenesis has been worked out, we immediately realize that there are different possibilities. In the first place, parthenogenesis could occur without a reduction-division, as seen in aphids and other forms. Or parthenogenesis could be started after a reduction-division by secondary fusion of the egg-nucleus and the reduction-nucleus, as has been shown for *Artemia* and the starfish. Or, thirdly, an apparently normal formation of the polar bodies could occur, but without reduction of the chromosomes, caused by their failure to conjugate, as has been shown for *Nematus* (Doncaster, 1906) and *Rhodites* (Schleip, 1909). Finally, it is possible that, after normal reduction-divisions, the diploid number is restored before segmentation by a rudimentary division. No case of this kind has been proved beyond doubt.

The current ideas about the relation of parthenogenesis and sex are primarily concerned with the chromosome number and with an eventual extrusion of a single sex-chromosome. If these conceptions are valid, the different methods of reaching the diploid number of chromosomes would not affect the resulting sex. But, at the same time, these conceptions have failed to explain why parthenogenesis produces only males or only females or both sexes, and that sometimes with, sometimes without, reduction. The ideas about sex-determination which we have developed during the last few years enable us, as we believe, better to understand the different facts about parthenogenesis and to fit them into the general scheme of sex-inheritance.

Since we stated our views in a general way not long ago (Goldschmidt, 1916), we do not need to repeat them here in extenso. We might mention only that we believe to have proved (1) that there are different sex-factors for the sexes, both acting independently in both sexes; (2) that both factors exhibit a definite quantitative action; (3) that the definitive sex depends upon which factor has the higher value, or, expressed in a formula, $F - M > e = ♀$, $M - F > e = ♂$; (4) that one of these factors is carried in the sex-chromosome, the mechanism of their distribution—or, in Mendelian symbolism, of the gamete-formation in heterozygosis—being the means of regulating the values for e in favor of F or M respectively; (5) that the factor not carried in the sex-chromosome, namely F in the case of female heterozygosis, M in male heterozygosis, is inherited maternally, probably in the protoplasm of the egg.

Thus the conclusions which we must draw concerning the relation of parthenogenesis and sex are of course different from those of older writers. Let us first glance at the possible combinations to be derived from our conceptions. For convenience we use the formulæ: $(FF)\ Mm = ♀$; $(FF)\ MM = ♂$, in the case of female heterozygosis, and $(MM)\ Ff = ♂$, $(MM)\ FF = ♀$, in the case of male heterozygosis. And we keep in mind the fact that the factors within the brackets are inherited maternally and are, therefore, contained in every egg, the others being carried by the x-chromosomes and following their distribution. The possibilities are now as follows:

1. Female heterozygosis. ♀ = (*FF*) *Mm*, ♂ = (*FF*) *MM*.
   - *A*. Parthenogenesis occurs with the reduced number of chromosomes. Offspring must be female as no set *MM* can be produced.
   - *B*. Parthenogenesis occurs with normal number of chromosomes in consequence of no reduction-division taking place. All offspring are female, as the maternal combination is preserved.
   - *C*. Parthenogenesis occurs with the normal number of chromosomes, reached by readjustment after reduction.
     - *a*. Readjustment accomplished by conjugation of egg and polar nucleus. All offspring female, since maternal combination remains.
     - *b*. Readjustment accomplished through rudimentary division before cleavage. The reduction had led to eggs with *M* and eggs with *m*. *M* eggs then become *MM*, *i. e.*, males, *m* eggs become *mm*, *i. e.*, females, if viable at all.

Conclusion.—Parthenogenesis with female heterozygosis can result in the production of (*a*) females exclusively (cases *A*, *B*, *Ca*); (*b*) males exclusively (case *Cb* when *mm* eggs not viable); (*c*) both sexes (case *Cb* if all eggs are viable, or any combination of *Cb* with the other cases).

2. Male heterozygosis. (*MM*) *FF* = ♀; (*MM*) *Ff* = ♂.
   - *A*. Parthenogenesis occurs with the reduced number of chromosomes. Offspring nothing but males. However, the occasional formation of females is possible when a case of non-disjunction occurs, leaving both *FF* inside the egg.
   - *B*. Parthenogenesis occurs with the normal number of chromosomes in consequence of the failure of reduction. The maternal combination being preserved, all offspring are female. In this instance males can be produced if one *x*-chromosome is extruded during the equation division.
   - *C*. Parthenogenesis occurs with the normal number of chromosomes reached by readjustment after reduction.

a. Readjustment through conjugation of egg and polar nucleus. The maternal combination being preserved, only females are produced.

b. Readjustment through rudimentary division before cleavage. Only female offspring result, since every egg contains *FF*. In case of non-disjunction, an exceptional male may appear, provided an *ff* egg is viable.

Conclusion.—Parthenogenesis with male heterozygosis can result in the production of (*a*) females exclusively (cases *B*, *C*), exceptional males explained by *Cb* or the occasional occurrence of *A* or *B;* (*b*) males exclusively (case *A*), exceptional females explained by non-disjunction or the occasional occurrence of *B*, *C;* (*c*) both sexes (case *B* or combination of *A* with *B* or *C*).

We may now, by surveying briefly the facts known about parthenogenesis, show that the above explanation holds good for all of them.

1. *Hymenoptera.*—The classic case of the bee is of special interest because it demonstrates the possibility of sex-differentiation without the use of the usual method of the formation of two kinds of gametes. It is important also because it shows that we are entirely at a loss if we express ourselves in Mendelian symbols without referring to the cytological facts. Parthenogenetic eggs produce males which develop with the reduced number of chromosomes.[1] We are concerned with case 2*A* of the series above described. In spermatogenesis no reduction occurs and only one kind of spermatozoa is formed, being in constitution identical with the ripe egg. Every fertilized egg, therefore, develops into a female. Occasional females derived from parthenogenetic eggs—reported from time to time—(the same for ants)—can be explained by non-disjunction (2*A*) or the occasional occurrence of 2*B*, *C*. Occasional males from ferfertilized eggs are also possible if a non-disjunction egg (with both *FF* in the polar body) is fertilized.

The other hymenoptera show no difference in principle. Where parthenogenesis results in the formation of both males and females, the former develop with the haploid number of chromosomes (case 2*A*), the latter with the diploid number (2*B*). Spermatogenesis and fertilization are the same as in the bee (*Neuroterus* according to Doncaster, '10). If parthenogenesis results in female offspring, development occurs with the diploid number, no reduction taking place in spite of two maturation divisions (*Nematus*, Doncaster, '09, *Rhodites*, Schleip, '09). Occasional males as in 2*A* or 2*Cb*.

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[1] For cytological facts see Nachtsheim, H. ('13).

2. *Rotatoria.*—The relation of parthenogenesis to sex seems to be exactly the same as it is in Hymenoptera. (Lauterborn, '98, Shull, '10, Whitney, '09). Parthenogenetic eggs without reduction give females, with reduction males, the latter if fertilized, females. We must suppose that the spermatogenesis is similar to that of the bee.

3. *Aphids.*—The well-known work of Morgan ('09) and von Baehr ('09) shows them to fall into case *B*.

4. *Phasmids.*—Their behavior is not yet clear, either experimentally or cytologically. Probably they behave like some gall-wasps with occasional males (see von Baehr, '07).

5. *Lepidoptera.*—The group of the Psychidæ, which exhibits regular parthenogenesis, is cytologically most interesting, as will be shown in a paper by Dr. Seiler now in press. The results seem to fit our conceptions. About the cases of facultative parthenogenesis as described here we know only that both sexes are produced and contain the diploid number of chromosomes. We must suppose that we are concerned with case 1*Cb* or 1*Cb* combined with 1*B* or 1*Ca*.

6. *Ostracoda and Cladocera.*—Although these groups are greatly favored by experimentalists, we know comparatively little about their cytology (Woltereck, '98, Schleip, '09, Kuehn, '08). It is possible that they belong to the same group as the Rotatoria, but in *Ostracoda* parthenogenetic female-producing eggs undergo no reduction division. The experimental results in sex-production in this group make it seem possible, however, that we are here concerned with something quite different. This possibility will be discussed on another occasion.

7. *Artemia.*—The most recent writers on the subject (Artom, '11, Fries, '09) agree that the parthenogenetic races develop with the diploid number of chromosomes, the bisexual races in

the usual way. Nothing is known about the cytology of the occasional males in the parthenogenetic races. It is therefore impossible to tell with which of the possibilities we are here dealing.

8. *Nematodes.*—The strange type of parthenogenesis described for a Rhabditis by Krueger ('12) and resulting in female offspring, occurs without a reduction of the chromosomes. Here we have case 2*B*.

9. *Echinoderms.*—(Artificial parthenogenesis.) Tennant's work makes it pretty certain that the male is heterozygous. Artificial parthenogenesis with reduced chromosome number ought to yield males as in the bee. This has been found to occur in the few recorded specimens.

10. *Amphibia.*—(Artificial parthenogenesis.) We do not know which sex is heterozygous in Amphibia. R. Hertwig thinks it is the male. We tried ('11, '13) to show that the experimental results favor the view of female heterozygosis. Moreover we do not know whether parthenogenesis takes place with the haploid or the diploid number of chromosomes. The expectations can therefore fit any of the above enumerated cases.

I want to emphasize, finally, the fact that our thesis, if expressed in terms of cytology, is nothing but Wilson's old hypothesis of the one portion-two portion *x*-substance. But our experiments have allowed us to give a physiological meaning to this conception and to bring this cytological conception into harmony with Mendelian formulations.

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BIOLOGICAL BULLETIN

REACTIONS OF AMEBA TO LIGHT AND THE EFFECT OF LIGHT ON FEEDING.

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CONTENTS.

Introduction 45
Experiments with Light 49
  White light 49
  Red light 50
  Blue light 53
  Violet light 56
  Green light 57
  Yellow light 58
  Orange light 58
Experiments with dark beams 59
Reactions toward solid particles when stimulated at the same time by beams of light or of darkness 62
Conclusions 67
Summary 70
Bibliography 71
Explanation of plates 74
Table of abbreviations 74

INTRODUCTION.

The reactions of ameba-like organisms to light have been studied by a number of investigators in order to determine whether "naked" protoplasm is capable of responding to light waves. Whenever intense light was used as a source, amebas, pelomyxas and plasmodia reacted negatively, and as a consequence it was tacitly assumed that in general naked, "undifferentiated," protoplasm reacted negatively to light.

But speaking now only of experiments performed on amebas, for I do not wish to leave the impression that in my opinion what is true of the behavior of amebas toward light holds also for plasmodia and pelomyxas, the first to be recorded appear to be those of Verworn ('89). He projected white light and various spectral colors of various intensities perpendicularly through the microscope slide, and observed no change of reaction as the ameba moved from one intensity to the other, or from one color to the other. With the experiment similarly staged, Davenport ('97) came to the same conclusions as Verworn. Amebas moved from a field of very weak light into one of very strong light, apparently without change of behavior, even when the change of intensity was sharp and abrupt. But Davenport showed that when a light beam is projected horizontally against an ameba, the ameba orients so as to flow away from the source of light. Harrington and Leaming ('00) showed that intense white, violet, or blue light, flashed on a moving ameba, arrested its movement momentarily; but red light was without any decisive effect. Mast ('10), experimenting under conditions similar to those under which Verworn and Davenport worked, confirmed Davenport's findings when horizontal beams of light are thrown against an ameba, and also concluded from his experiments that when an ameba finds a perpendicular beam of intense light in its path, it avoids the light in many cases. Mast also confirmed the general conclusions of Harrington and Leaming.

None of these investigators observed any but negative behavior, though Mast presumably looked for positive responses, for he says "I was unable to obtain positive reactions in *Stentor cæruleus*, *Amœba*, and fly larvæ" ('11, p. 270). The reason Mast failed to get positive responses was because the beams of light which he used were too large and the light was too intense. And further his apparatus was perhaps defective. He says: "The beam of light was produced by focusing a limited area of a luminous Welsbach mantle on the slide by means of *the mirror* and an Abbe condenser" ('11, p. 78). If he used an ordinary microscope mirror, as he seems to have, a very faint subsidiary beam as well as the very intense primary beam, was projected through the slide. The one is produced, of course, by the front surface of the glass, and is very readily overlooked; the other is produced by the surface of the silvering. Since the subsidiary image shows only on one or two sides of the main image, if square, the chances are about even that the ameba came into contact with the subsidiary image first, and therefore the likelihood of a positive response is increased. From my own experience I have found that whenever mirrors are necessary, it is absolutely essential that they are silvered on the front surface; otherwise subsidiary images will result, and in case monochromatic spectral light is used a considerable degree of impurity may occur. If Mast used back surface mirrors in his work on the light reactions of ameba, some of his experimental results are, therefore, incompletely described and consequently inconclusive; and his inability to observe positive responses was due, in part, to improper staging of the experiment for this purpose.

The great majority of the light experiments in this paper show positive reactions; a few are indifferent, and a few negative. This proportion resulted from the manner in which the experiments were set, and from the fact that observations were based upon the behavior of amebas *before* they came into contact with the beam of light as seen by the eye, instead of after, as previous observers did. But in a great many cases negative behavior did not result even after the ameba came into contact with the light; but if the beams had been larger it is not improbable that the proportion of negative reactions would have been larger.

These experiments on the reactions of ameba to light were performed not only for the purpose of testing the sensitiveness of these organisms to light by itself, but especially to see whether differences in intensity, quality or direction of light rays are capable of causing changes in the behavior of amebas while feeding. In order to let the results speak as definitely as possible, a number of experiments were performed first with beams of light only as stimuli. Later, particles of food were presented in connection with the beams of light. By comparing these two sets of experiments with each other and with the results of previous experiments on the feeding habits of ameba (see Bibliography for references) the effect of light on feeding may be readily observed.

Several investigators, as already pointed out, made observations on the reactions of ameba to light, but all of them made use of large areas of very intense light. But for purposes of comparison with other sources of stimuli it was thought essential, in my own work, to reduce the areas of light to a size comparable with the size of food and other objects which were placed at the disposal of the ameba. By throwing the beams of light vertically through the slide on which the amebas were placed, the area of the cross section of the beam of light used for stimulating the ameba could be varied within the desired limits.

As sources of light the Welsbach gas mantle and the Leitz Lilliput arc were used. The gas light was passed through five cm. of distilled water containing a very small quantity of an ammoniacal solution of copper sulphate to absorb the excess of green and yellow rays and the distinctive heat rays. Between the water and the microscope was interposed an opaque screen with several small, clear-cut pinholes in it. By moving the screen away from or toward the microscope, and focusing with the substage condenser, the size of the projected beams of light through the microscope could be easily controlled. The ordinary mirror of the microscope was discarded and a front surface mirror placed in its stead, in order to avoid reflection from the front surface of the glass and so producing a subsidiary image on the slide. The great sensitiveness of ameba to light makes this precaution absolutely necessary.

When spectral light was used, the arc was employed as a source, and either prism or grating interposed between clear distilled water and the screen.

The amebas were placed on large, clear thin cover glasses in clear culture fluid, without a cover glass over them. Usually in these light experiments the beams of light were left stationary while the coverglass containing the ameba was shifted, whenever shifting was necessary for experimental purposes. Both intermittent and continuous beams of light were employed. Intermittent light was produced by moving an opaque object up and down between the screen and the microscope. In a general way, continuous and intermittent light had about the same effect on the ameba. After orientation, it is worth noticing, the ameba advanced as definitely and as uniformly toward intermittent as toward continuous light.

The work was done in a dark room in which there was very little diffuse light. Some light was necessary in order to make the camera lucida drawings illustrating the experiments. But this light was confined, as far as possible, to the paper on which the drawings were made, and no more light was used than was necessary. Extra precautions were taken to prevent any but vertical beams from reaching the ameba.

Experiments with Light.

White Light.—Two beams of white gas light were projected in front and to the right of an *Amæba dubia* flowing along in spatulate form—Fig. 1. The ameba flowed straight past the small spot of light and also passed the larger beam a short distance, when two side pseudopods were thrown out directly toward the larger beam. The pseudopod nearer the light spot enlarged until it had flowed over the light, then it was arrested and the other pseudopod became the main one through which the ameba moved away. The slide was then moved so that the small beam lay in front of the ameba—Fig. 5. Two pseudopods were formed on the left side. The main pseudopod moved into contact with the small beam of light, then sent out on the left a pseudopod which moved directly into contact with the larger beam. At the same time the more posterior of the previously formed pseudopods sent out on its right a pseudopod which also moved into contact with the larger beam while the more anterior pseudopod was withdrawn, but this pseudopod was finally withdrawn as the one which previously moved into contact with the light, moved on over the light spot—8. These experiments show very clearly that small beams of white light attract amebas before they actually come into contact with the beams.

An *Amœba proteus*[1] was placed so that a small beam of white light lay to the right of its path—10. Two pseudopods were thrown out, one on the left and one on the right, but both were quickly retracted. The tip of the ameba then turned sharply to the right and directly toward the light—14. The ameba then moved forward in the original direction for a short distance

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[1] The name *Amœba proteus* of Pallas and Leidy as used in this paper also includes the species *A. discoides* Schaeffer, discovered to be distinct from *A. proteus* after the experimental work recorded in this paper was done (for preliminary descriptions see Schaeffer, '16*b*).

—16. Finally a pseudopod was thrown out on the right partly encircling the light—19. After breaking up into a number of pseudopods the ameba moved away. The ameba was shifted with the beam of light on the left—22. Movement became somewhat uncertain at first, then streaming was reversed—25. As the ameba moved forward in the new direction, it gradually turned to the right until it was flowing directly toward the beam of light—29. After partly surrounding the beam—35—the ameba moved away.

Summary.—From the foregoing experiments it is clear that ameba responds positively to white light under the given conditions. The ameba is attracted from a distance of sixty-five microns or more. Since the beam is projected vertically, the question at once arises: How does the ameba become aware of the beam of light? Is some light reflected horizontally by particles of solid matter in the water; or is some of the light energy transformed into heat or other form of energy which, being radially propagated, stimulates the ameba? These are very important questions in sense perception, but they must remain unanswered for the present. The light apparatus at my disposal was too crude to attempt a solution of them.

In most cases the ameba reacts positively until it comes into contact with the beam of light, when negative behavior usually sets in. This difference in behavior may be due to differences in the intensity of the stimulus. Both *proteus* and *dubia* react positively to white light.

It is quite clear from these experiments that light of the intensity used does not tend to inhibit directly the formation of pseudopods, nor does it seem to have any other direct effect on the movement or form of ameba.

Red Light.—The apparatus for producing monochromatic light of the various wave-lengths was a Leitz Lilliput arc light, fifteen cm. of distilled water, a piece of grating with a slit two millimeters wide, a screen of heavy drawing paper with a pinhole about one half mm. in diameter with clear-cut blackened sides, front surface mirror and condenser. The screen was blackened on the far side and lighted on the near side with just enough light from a Welsbach mantle to barely see the amebas. The spectrum on the screen was about two cm. long. By means of the pinhole therefore only about one fortieth of its length was projected at any one time. The apparatus was rather crude and somewhat unsatisfactory, but it seemed to be sufficiently reliable for exploratory work.

On account of the impracticability of testing spectroscopically the beams of light which were thrown into the microscope, no accurate values in wave lengths can be given for blue, red, yellow, etc., so there may have been, for example, a few "rays" of orange or green in the yellow when that color is specified; but such mixtures cannot have been very significant since the size of the pencil of light was very small, roughly one fortieth of the length of the visible spectrum. As a matter of fact some rays of all wavelengths were mixed with the pencil of monochromatic light, but this defect could not be remedied, for some diffuse light (though this of course might be monochromatic spectral light) is necessary in order to see what is going on. But if any change in behavior takes place with reference to a pencil of monochromatic spectral light, the change must be caused by the more intense monochomatic light, since the diffused white light is equally intense all over the field.

A small beam of red spectral light was projected in the path of a *proteus*—37. The ameba moved forward a short distance, then sent out a pseudopod on the left—40. From this pseudopod another was sent out on the right—45—which moved directly toward and over the red beam. The pseudopod was soon withdrawn and the ameba then moved on.

Another *proteus* was brought into view with the light on the right—49. The ameba turned sharply to the right, then to the left, and finally moved over the red light. Presently a pseudopod was thrown out on the right through which the ameba moved away.

In the next experiment the light was placed on the left of a *proteus*—53. The ameba moved forward for a short distance, then threw out a pseudopod on the left—56—through which the ameba moved off without coming into contact with the light. The ameba was then shifted with the red beam lying on the left —59. As the ameba flowed forward toward the light spot a pseudopod was thrown out on the right, through which the ameba finally moved off. The ameba was then shifted so that the light lay on the right—63. The ameba turned to the left and moved on. The ameba was again shifted with the light lying on the left—65. As the ameba moved forward a small pseudopod which was thrown out on the left moved directly into contact with the light. The next four experiments—69–93—all show that the beam of light was sensed before the ameba came into contact with it, and also that the light produced more or less definite positive behavior, followed by indifferent or negative behavior.

A beam of red light was projected in the path of another *proteus*—94. A pseudopod was thrown out toward the light. After it came into contact with the light—97—the ameba moved away through a pseudopod thrown out on the left—99. The ameba was then shifted with the beam of light on the left—101. The ameba moved forward, then turned slightly to the right—104. A small pseudopod was then sent out toward the red light—107—but after covering half the distance it was retracted while a pseudopod was thrown out on the same side, but further forward. From this pseudopod still another was sent out on the left. The ameba thus partly encircled the red light. The ameba was shifted again with the red light lying in front of it—111. The ameba threw out a pseudopod on the right and passed on—111–113—but when the tip of the main pseudopod extended beyond the light, it broke up into four pseudopods—115—of which the left posterior ultimately became the main pseudopod through which the ameba moved away.

Another *proteus* was then brought into the field with a beam of red light lying to the right—120. The ameba moved on a short distance, then threw out two pseudopods on the right—122—one of which moved into contact directly with the red light—125—but which was retracted as the ameba moved on. The ameba was then shifted with the light lying on the left—125. Pseudopods were sent out on both sides but the one on the right finally became the main one leading the ameba away. The ameba was again shifted with the red light lying on the right—128. A pseudopod was thrown out on the right directly toward the light. When it came into contact with the light it forked, the limbs moving forward with the light spot between them —133. The right limb became the main pseudopod through which the ameba moved off.

A beam of red light was projected to the right of another *proteus*—167. The tip of the ameba turned slightly toward the left, then resumed its original direction. The ameba was then shifted with the red light on the left—170. There was at first a tendency for pseudopods to form on the right, but, as the ameba moved forward, two were formed on the left in the region of the light. One of them moved a considerable distance toward the light—174—but was then retracted as a pseudopod on the right was thrown out to become eventually the main pseudopod. The ameba was then shifted with the red light lying on the left—176. As the ameba moved forward a large pseudopod was thrown out on the left, directly toward the light. This pseudopod became the main one through which the ameba moved away.

Summary.—Red spectral light produces about the same changes in behavior as white light. The vertical beam of red light is sensed at a distance, and in almost all cases produces positive behavior. In some few cases an ameba may behave indifferently or even negatively; but if the experiments are repeated several times a positive reaction is almost sure to occur. Amebas are therefore not negative or positive permanently with respect to beams of red light, but the behavior may readily change from the one to the other aspect. Red light of the intensity used does not seem to stimulate the ameba disagreeably when it moves into direct contact with the beam of light, for in a number of cases the ameba moved on over the light without visible change of behavior. In some respects the ameba tended to encircle the source of light in the same manner in which it sometimes encircles solid objects.

Blue Light.—A beam of impure[1] blue spectral light was projected to the right of a *proteus*—135. The ameba turned to the left and moved away, a decided negative reaction. The light was shifted so that it lay directly ahead of the ameba—139. The ameba threw out three pseudopods on the left, but almost immediately retracted them. Another was sent out on the right, but it also was soon retracted. The ameba then moved forward, and in passing the blue light sent out a little pseudopod toward it—143. The ameba finally turned to the left and moved on. The ameba was shifted again with the blue light on the right—145. After the tip of the ameba had passed by the blue light it turned to the right. A small pseudopod was also sent out toward the light—148. The ameba was then shifted with the blue light lying directly in front—150. As the ameba moved forward a small pseudopod was thrown out into contact with the light—152. Then the ameba moved on. The ameba was shifted again with the blue light directly ahead—154—but a decided negative reaction set in. But when shifted again with the blue light ahead—158—the tip of the ameba turned away from, and then toward the light, but finally moved on in the original direction. The ameba was shifted again with the blue light directly ahead—162. The resulting behavior was indefinite.

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[1] Between the grating and the arc was placed twenty cm. of distilled water containing a very little ammoniacal copper sulphate. The copper salt gave rise to a subsidiary faint yellowish spot of light not quite coinciding with the blue. The yellow image disappeared when the copper salt was omitted from the distilled water. The yellow image was probably due to fluorescence of the copper sulphate.

Another *proteus* was then brought into the field with a beam of blue light directly ahead—181. The ameba then threw out a pseudopod on the left and from this one another on the right, and from this last one still another on the right, so that the light was partially encircled. The ameba then moved off through a pseudopod on the left. The ameba was shifted with the blue light on the right—186. A pseudopod was thrown out on the right directly toward, and into contact with, the beam of light. The ameba then flowed away through another pseudopod on the right. The ameba was again shifted with the light lying on the right—190. Two pseudopods appeared on the right, one of which was directed toward the light spot. Both pseudopods were presently retracted and the ameba moved on to the left. Very peculiar behavior was observed when the ameba was shifted again—194. The behavior was at first negative, the ameba moving away to the right—195—but streaming was then reversed, and as the ameba passed by the light, two small pseudopods were sent out toward it—200. They were retracted however as the ameba moved on. When shifted again with the blue light to the left—201—the ameba turned toward the light and then passed on to the left. The ameba again reacted positively when shifted with the light straight ahead—204. When shifted again the ameba reacted positively but rather uncertainly—209. In the next experiment, with the beam of blue light on the left, negative behavior was induced—216.

A beam of pure (see footnote p. 11) blue spectral light was projected to the right of a *dubia*—219. The ameba moved past the light spot for a considerable distance without any change in behavior. Then two pseudopods were sent out: one directly toward the light, and the other near the tip, but also on the right side—220. As the pseudopods enlarged, the tip of the ameba also turned sharply to the right—221. When the posterior pseudopod came into contact with the light, the pseudopods on the right were retracted, and two others thrown out on the left —222—but these also were retracted after a few seconds, and the ameba then moved on in the original direction. The ameba was then shifted with the blue light lying directly ahead—223. The tip of the ameba (only the tip of the ameba is shown) turned to the left—224—but a pseudopod was thrown out on the right toward and into contact with the light—225. The ameba flowed partly over the light—226—but withdrew from it later and moved off through a pseudopod on the right.

Summary.—There is no marked difference between the reactions toward red light and those toward blue. Blue light induces positive behavior in as marked a degree as red, though when all the experiments are considered, red light seems to be somewhat more attractive than blue. Blue light, like red and white, induces both negative and positive reactions. Blue light can also be sensed at a distance.

The experiments with the *dubia*—219–222—are interesting inasmuch as a pseudopod was thrown out at the tip of the ameba on the side on which the light lay, some time after this part of the ameba had passed the light. It may be noted also that the tip of the ameba turned strongly in the same direction. It appears quite unlikely that the light acted as an efficient cause on this region of the ameba at the time of the formation of the pseudopod, for the effect of the light was continually decreasing in intensity as the ameba moved away from it. The light would therefore be expected to have the maximum effect at maximum intensity, which was when the tip of the ameba was closest to the light. It is improbable therefore that the throwing out of a pseudopod and the bending of the tip to the right were caused by the impinging of the light rays at that region at that time. It is possible that this behavior is the result of the cumulative effect of the light rays while the ameba was passing the beam. There was formed a tendency toward a positive reaction some few seconds before it expressed itself in visible change of behavior, and when this tendency "came to a head" it resulted in exaggerated behavior; for two pseudopods were thrown out on the stimulated side and at the same time the tip of the ameba was turned to the right. This feature of ameban behavior—the formation of two pseudopods on the stimulated side, one near the anterior end and the other opposite the stimulating object after the tip of the ameba has passed the stimulating object—is frequently observed and is of great interest. It indicates several things. First, it effectively disposes of the hypothesis that the movement of pseudopods toward an object is directly induced by the object. Second, it shows that there is some sort of a coördinating or integrating agency at work in the ameba so that the larger part of it, at least, tends to react in a coördinated manner, even if there are separate centers of reaction. When the posterior pseudopod came into contact with the light, negative behavior set in suddenly. The pseudopods on the right were promptly withdrawn and two others were rapidly projected on the left. Nevertheless, the ameba finally moved on in the original direction.

Violet Light.—The violet light that was selected was as near to the end of the visible spectrum as possible. A beam of violet light was projected on the right of a *proteus*—230. A small pseudopod was thrown out on either side—232—the one on the right being directed toward the light. The ameba moved away however through the pseudopod on the left. The ameba was shifted with the light again on the right—235. The tip of the main pseudopod turned to the right and moved into contact with and then on over the light. The ameba was shifted with the violet light on the left—241. As the ameba moved forward past the light a small pseudopod was sent out toward the light, but it was withdrawn before it came into contact with the light.

When shifted again with the light on the right—245—the ameba sent out a pseudopod anterior to the light—249—but it curved backwards toward the light as the ameba moved forward—251. On the next trial—253—the ameba first turned away from the light then sent out a pseudopod directly into contact with it; then another pseudopod was sent out on the side and anterior to this one.

Summary.—Amebas react positively, negatively, or indifferently toward violet light. The greater number of changes of behavior produced by violet light were positive. No definite differences could be observed between the effects of violet light and those of any other spectral light thus far described.

Green Light.—A beam of green spectral light was projected to the right of an *Amœba dubia*—259. As the ameba moved forward the tip of the main pseudopod moved to the left. A small pseudopod was formed on the right toward the light. The ameba then turned toward the right and at the same time threw out a pseudopod on the right near the tip of the main pseudopod. Both pseudopods were withdrawn as the ameba moved on. (Compare the behavior of this ameba with that illustrated in Figs. 219–222.)

The beam of green light was then projected to the right of a *proteus*—262–265. As the ameba moved forward, a large pseudopod which was thrown out on the left, was soon retracted, the ameba moving on in a straight path. The ameba was shifted—266–270—with the green light to the right. As the ameba moved on past the light, a small pseudopod appeared on the right near the light, but it was retracted before it had developed to any extent, as the ameba flowed on.

These few experiments indicate that the effect of green spectral light is similar in a general way to that of white, red, blue, etc. Although the positive reactions in these experiments are slight, they are nevertheless definitely positive. If I had made as many experiments with green light as with red or blue, I have no doubt that more decided reactions would have been obtained.

Yellow Light.—A beam of yellow spectral light was projected straight ahead of a *proteus*—271. The ameba moved on without any definite change of behavior and passed over the beam of light. The change in the direction of movement—275–277—indicates that the yellow beam had a disagreeable effect after the ameba came into contact with it. When the ameba was shifted—278—the tip of the main pseudopod turned to the left—a negative reaction continued from the previous experiment. But while passing the beam of light the negative condition gave way to a positive as is shown by the turning of the tip of the ameba toward the light—282. A pseudopod was then thrown out on the left on the convex side, and from this one another on the left through which the ameba moved on, again a negative reaction. The ameba was shifted again—285—with the yellow light ahead. The ameba turned sharply to the right, but as it passed by the light a pseudopod was thrown out on the left directly toward the light—287. This pseudopod became the main one through which the ameba flowed on over the light. As the ameba came nearly into contact with the light, a pseudopod was thrown out on the right—290—an indication of a negative reaction, but it was soon retracted.

To summarize: Amebas respond positively, negatively or indifferently to beams of yellow spectral light. As far as my experiments go, yellow light has about the same effect as red or blue or the other spectral colors which have so far been considered.

Orange Light.—A beam of orange light was projected to the left of a *proteus*—294. The ameba turned to the left and moved directly into contact with the light. When the ameba came into contact with the light, a pseudopod was started on the right, but it was soon retracted and the ameba flowed on over the light without further change of behavior. The ameba was then shifted with the orange light on the left—301. The tip of the ameba turned to the left, then broke up into two pseudopods of which the left one turned still further to the left and finally became the main pseudopod through which the ameba flowed away. The ameba was shifted again with the orange light slightly to the right—306. A pseudopod which was thrown out on the right elongated as it turned to the left. When the tip of the pseudopod had passed the light, a new pseudopod was thrown out on the left near the light—309. This pseudopod moved straight forward for some distance, when another pseudopod was sent out on the left—312—but this one was finally retracted as the ameba moved away.

Orange spectral light induced positive reactions in the ameba of this series of experiments, though they were wholly positive only in the first experiment. In the other experiments the tendency was toward positive behavior, but the source of stimulation was not definitely sought. The beam of light attracted the ameba only mildly after the first encounter, and the tendency to move forward (Schaeffer, '14*a*) may be presumed to have been about as strong as the tendency to move toward the beam, hence the partial encircling of the beam in the last two experiments. In my laboratory notes there is recorded one experiment with orange light in which the behavior was wholly negative.

Experiments with Dark Beams.

When it was seen that white light and spectral light of various wave-lengths had essentially the same effect on ameba, it seemed likely that these results were due to differences in intensity between the beam of light and the diffuse light on the field. The suggestion then presented itself whether a decrease in intensity of light in a small area produces a similar result. A dark beam was therefore projected into the microscope. The source of the dark beam was a hole in the screen, leading into a blackened light tight box fastened to the back of the screen. The sides of the hole were blackened to prevent as far as possible the reflection of light. The rest of the screen was illuminated by diffuse light, as in the other experiments, but more brightly so as to increase the contrast between the field and the hole. The hole as viewed through the microscope appeared as a very dark gray spot.

A *proteus* was shifted so that the dark spot lay directly ahead of the ameba—314. The tip of the ameba broke up into two pseudopods, one of which turned to the right and the other to the left of the dark spot, indicating a negative reaction. As the right pseudopod moved forward it turned to the left until it came into contact with the dark spot—a positive reaction. The ameba then moved on through this pseudopod and partly over the dark spot. The ameba was again shifted so that the dark beam lay directly ahead—319. A pseudopod which was thrown out toward the right led the ameba away, a definite negative reaction.

Another *proteus* was then brought into the field with the dark spot directly ahead—322. The ameba turned to the right and moved on, avoiding the dark beam. The ameba was then shifted with the dark spot straight ahead—325. The ameba became irregular in its streaming at the anterior end, indicating that the ameba sensed the dark spot and that there was present a tendency to react negatively; but the tendency to negative reaction was weak, for the ameba started presently to move over the dark area. The ameba, in irregular shape, was shifted again with the dark spot directly ahead—329. When the ameba came into contact with the outer edge, the tip of the main pseudopod forked, the right prong becoming the main pseudopod through which the ameba moved away. Negative behavior is again shown here. The ameba was shifted with the dark spot directly ahead—333. A pseudopod was thrown out on the left as the ameba moved into contact with the dark spot—334—indicating a tendency to negative reaction, but it was withdrawn as the tip of the ameba proceeded for some distance beyond the further edge of the dark area—336. The ameba was moved again so that the dark spot lay slightly to the right—340. The ameba moved into contact with the dark area, then sent out a pseudopod on the left, but it was soon withdrawn and at the same time another was sent out on the right. The ameba finally moved on in the original direction. Here we have first positive behavior in the turning of the ameba toward the dark spot; then negative behavior in the formation of the pseudopod on the left; then again positive behavior in the resumption of forward movement and the formation of the pseudopod on the right. In the next trial the ameba was moved with the dark spot slightly to the right—346. The ameba turned slightly further to the left, then directly toward the right and toward the dark spot—348. When the ameba came into contact with the dark spot, a pseudopod was thrown out on the left, but it was withdrawn as the ameba moved on.

Another *proteus* was then brought into the field with the dark spot slightly to the left—354. The tip of the main pseudopod broke up into two pseudopods of which one moved directly toward and over the dark area. But when the ameba came into contact with the dark spot, a large pseudopod was thrown out on the right—356—but it was withdrawn as the ameba moved on over the dark spot. The ameba reacted in effect positively throughout the experiment, but a strong tendency to react negatively is shown by the breaking up of the main pseudopod into two pseudopods—355—and by the appearance of the pseudopod on the right when the ameba came into contact with the dark spot—358. The ameba was then shifted with the dark spot slightly to the left—359. The ameba moved forward a short distance, then the tip of the main pseudopod spread out, and then the protoplasmic stream was suddenly reversed and the ameba moved away to the right through a vestige of a previous pseudopod—a decided negative reaction. But the ameba was then moved with the dark spot directly ahead—363. After the ameba had moved forward a short distance the tip forked broadly, and the ameba moved off through the left prong, again a decided negative reaction.

Summary.—Amebas become aware of dark spots before they come into contact with them, as seen through the microscope with the eye, just as they become aware of beams of light before encountering them. In most cases the tendency is to react negatively, but in some instances the first change in behavior is positive. Usually when the ameba first comes into contact with the dark beam there is a tendency toward negative behavior, as is shown by the formation of pseudopods which, if they became main pseudopods, would lead away from the dark area. These pseudopods are usually withdrawn as the ameba moves forward over the dark spot. The behavior is seldom wholly positive or wholly negative; in most cases there is some vacillation between negative and positive reactions. The reactions on the whole were not so pronounced as those toward light. The actual stimulating quality is very likely to be looked for in the difference in light intensity between the dark spot and the field.

Reactions toward Solid Particles when Stimulated at the Same Time by Beams of Light or of Darkness.

A grain of globulin was placed over a small beam of blue spectral light, and arranged so that the illuminated globulin lay in the path of an *Amœba proteus*—365. The ameba moved in spatulate form directly toward the globulin-blue light until it came into contact with the globulin, when a pseudopod appeared on the right. The ameba however ingested the globulin in typical manner and then quieted down over the blue light for over twelve minutes.

A grain of globulin was placed in the path of a *proteus* with a beam of green spectral light between the ameba and the globulin —373. Through a pseudopod thrown out on the right the ameba moved away from the light-globulin—374. The ameba then broke up into four pseudopods of which the left one of the middle pair became the main pseudopod. The ameba moved forward through this pseudopod toward the globulin in a curved path, apparently, so as to avoid the light, pushed the globulin ahead a short distance, and then ingested it in an imperfect food cup. This is an interesting experiment. The beam of green light stimulated the ameba negatively when contrasted with the globulin. The ameba made a detour around the light to get to the globulin. This experiment should be compared with Figs. 1–13 in a recent paper (Schaeffer, '17*b*) in which very similar behavior is recorded as an ameba moved toward a grain of globulin with a grain of silicic acid lying immediately in front of the globulin.

A grain of globulin was placed to the left of a *proteus* with a beam of green spectral light between the globulin and the ameba —386. The ameba moved forward a short distance, then bifurcated, the right prong being directed backwards while the left prong was directed toward the globulin—388. The ameba moved toward the light at first—389—but presently the tip of the ameba broke up into two pseudopods. The one thrown out on the right enlarged rapidly as it moved in a slight detour around the light toward the globulin—392, 393. After rolling the globulin along the surface for a short distance it was ingested in a typical food cup. This experiment as well as the preceding, shows that a beam of green light acts as a disturbing factor when an ameba is stimulated at the same time by globulin.

A grain of globulin and a beam of yellow spectral light were placed to the right of the path of a *proteus* with the light between the globulin and the ameba—397. As the ameba moved forward it turned to the right and directly toward the yellow light. The ameba moved over the light, then turned to the left and moved into contact with the globulin, which the ameba rolled around a short distance before ingesting it in a normal food cup. The yellow light did not disturb the ameba when stimulated simultaneously by globulin.

A grain of globulin and a beam of yellow light arranged as in the preceding experiment were placed in the path of another *proteus* —408—but the behavior observed was negative, due doubtless to lack of hunger in the ameba. The ameba was in Y-shape at the beginning of the experiment—408. The ameba responded negatively by bending the right prong to the right and flowing along it. A pod was thrown out on the right, indicating the presence of a tendency to a positive reaction. The ameba was then shifted with the yellow beam straight ahead and the globulin a little to the left—412. The tip of the ameba forked, the axes of the limbs coinciding with the same straight line, and nearly perpendicular to the rest of the ameba—413. The right prong turned toward the yellow light—415—and presently two pseudopods were sent out a short distance toward the globulin—416 —but both were withdrawn as the ameba moved away through a pseudopod thrown out on the right—417. The ameba was shifted with the globulin straight ahead and the beam of yellow light to the left—419. The ameba moved forward a short distance when a pseudopod was thrown out to the left—421. This pseudopod became the main one, and after it flowed ahead some distance it turned to the right and moved toward the globulin —423. The tip of the ameba then turned to the right still more strongly and at the same time a pseudopod was thrown out in the direction of the stimulating objects—424. The posterior end now became activated but only for a short time—425. Several new pseudopods were formed indicating uncertainty in behavior, of which the one on the right extending toward the globulin became for the moment the main pseudopod—427. After moving toward the globulin for a short distance a pseudopod was thrown out on the right leading the ameba away from the test objects—429–432. Presently however the main pseudopod bent strongly to the left—433, 434—with the formation at the same time of two pseudopods on the convex side of the main pseudopod—434, 435. The pseudopod pointing toward the globulin moved forward a short distance—436—but this pseudopod was retracted as the other one of the two formed on the convex side became reactivated leading the ameba away from the globulin and light.

Since it was evident that the ameba reacted negatively to both light and globulin when these substances stimulated the ameba at the same time, two further tests were made on this ameba in which each test substance was used by itself.

The piece of globulin was laid some distance ahead of the ameba—438. The ameba moved toward the globulin a short distance then threw out a pseudopod on the left—440—through which the ameba moved forward with the globulin on the right—442. A pseudopod was then thrown out on the right toward the globulin while the tip of the main pseudopod turned strongly toward the left—444. When the pseudopod on the right came into contact with the globulin the main pseudopod was retracted—447, 448. The globulin was only partly surrounded—449. One of several pseudopods formed on the right led the ameba away, leaving the globulin behind.

After a few minutes the beam of yellow light was projected in front of this ameba—453. As the ameba moved forward several pseudopods were thrown out on either side of the main pseudopod, giving the ameba a very irregular shape. When the ameba came nearer the light—458—it advanced definitely forward passing the beam immediately to the right—459. The main pseudopod then swerved a little to the side as two pseudopods were formed on the right, through the lower one of which the ameba finally moved away from the light.

This ameba then reacted definitely positively to globulin but much less definitely positively to yellow light, when presented separately, but decidedly negatively when presented together.

The experiment shows that yellow light was the cause of the negative behavior when both it and globulin were presented together; or perhaps one ought to say yellow light, *in combination*, possessed deterring qualities which were absent when it stimulated the ameba alone. Here again we have another case of where the milder of two positively stimulating objects when encountered alone becomes negative when encountered simultaneously with the more strongly stimulating object (Schaeffer, '17*b*).

A dark spot with a piece of carbon lying in it was then placed in the path of a *proteus*—463. As the ameba moved forward it turned slightly to the left at first—467—but the tip of the ameba then turned to the right and moved directly toward the dark beam and carbon—471—until within about ten microns of the beam, when the ameba suddenly turned to the right—472. The ameba moved along in this direction without coming into contact with either the dark spot or the carbon. That this decided negative reaction was caused by the dark spot is evidenced by the next experiment in which the carbon was omitted—481. The ameba moved forward toward the dark beam for a short distance, then became very uncertain in its behavior. First it turned to the right, then formed a pseudopod on the left through which it moved forward with the dark spot on the right—484. A pseudopod was thrown out on the left but it was promptly retracted—487. At the same time the tip of the pseudopod turned very sharply to the right and moved directly toward the dark spot—488. The ameba continued moving toward the dark spot until within thirty microns of it—489—when the main pseudopod was retracted and the ameba then moved away to the left through another pseudopod which had been slowly forming while the ameba was moving toward the dark beam. A dark beam with a piece of globulin lying in its centre was placed to the right of the ameba with a pseudopod already turned toward the dark spot—492. The ameba flowed on through the pseudopod directly toward the dark spot. When within about eighty microns of it, the tip of the main pseudopod forked—495—indicating a tendency to negative behavior. The left prong which became the main pseudopod, moved directly forward until it came nearly into contact with the dark beam when a slender pseudopod moved into the dark area, and another, indicating a tendency to negative behavior, moved to the right —498. But when the ameba came into contact with the globulin the pseudopod on the right was withdrawn and then the globulin was ingested, the ameba quieting down over the dark spot. The experiments with this ameba show that the dark beam may be sensed at a distance of at least 150 microns, and that the globulin may be sensed as well apparently when lying on a dark spot as when illuminated.

A dark spot with a fragment of carbon lying on it was placed to the right of an ameba—503. There was no definite response. The ameba turned toward the right and moved toward the test objects in a more or less uncertain manner—504, 505. A pseudopod was then sent out on the left—506. After it had attained to considerable size it was withdrawn and the ameba moved off through a pseudopod thrown out on the right—508. The ameba was shifted with the carbon-dark spot lying on the right—509. As the ameba moved forward it turned toward the right—510—a small pseudopod being formed, as frequently happens, on the convex side, but a pseudopod was presently thrown out on the left—513—through which the ameba moved away. To show that it was the dark spot and not the carbon which produced the negative behavior, the results of the next experiment are appended—514. The ameba moved directly into contact with and on over the carbon without any sign of a negative reaction. A grain of globulin lying in a dark beam was then placed at some distance to the right of the ameba—520. An accidental jar displaced the globulin so that it lay near the anterior edge of the dark spot. The ameba changed its direction of motion and moved straight toward the globulin and the dark beam—521. (It may reasonably be doubted whether the ameba sensed the globulin at this distance.) The globulin was then moved to the further side of the dark beam—522. The ameba kept on moving forward for some distance, then its behavior became somewhat uncertain. The ameba moved slightly to the left—524. A pseudopod which was thrown out on the right—525—became later the main pseudopod. The ameba now moved directly toward the dark spot until within about twenty microns—528—when the protoplasmic current was reversed and the ameba moved off through a pseudopod on the left near the posterior end—529. Soon this pseudopod was withdrawn and the ameba flowed into another pseudopod (perhaps the vestige of the former main pseudopod) which led the ameba to the right (to the left of the dark spot)—533. After moving some distance in this direction, a pseudopod was thrown out on the left directly opposite to the dark spot—534. Two more pseudopods were then formed on the right through the more posterior of which the ameba moved on —535-537. A pseudopod was then formed on the left which led the ameba out of range of the dark spot—538. The ameba was then shifted with the dark spot on the right and the grain of globulin just in front of it—540. The ameba turned to the left—541—and sent out on the convex side a pseudopod through which the ameba moved forward with the dark spot on the left—543. As the ameba moved on it turned toward the dark beam but presently two pseudopods were thrown out from the middle of the ameba, one on either side—546. (The pseudopod on the left was evidently formed to enable the ameba to move at once into contact with the globulin; the one on the right was formed without assignable cause, although opposite pseudopods are frequently formed under conditions similar to these.) The pseudopod on the left moved through a curved path to the left into contact with the globulin—547-550. When the ameba came into contact with the globulin, it was pushed into the dark area. A slender pseudopod followed it while the tip of the main one remained stationary for the moment, which indicates that the dark area had a deterrent effect on the ameba—551. A food cup was however soon formed and the globulin ingested.

CONCLUSIONS.

From these experiments it may be concluded that white light and all the visible spectral colors cause positive responses; but whether all are equally attractive cannot be definitely stated, for experiments would have to be staged differently to produce accurate results. Nevertheless the red end of the spectrum seems to be somewhat more attractive than the blue.

What is of considerable interest in the behavior of ameba toward light is that the character of the response may vary rapidly. See Figs. 22 to 36; 37 to 48; and a number of other experiments. A negative reaction may be followed by a positive and vice versa. There is no definite relationship between ameba and light, on account of which the ameba is always either positive or negative or indifferent. Stimulation from light produces the same general character of reaction as stimulation from glass or carbon. The only observable difference is a quantitative one; light beams are sensed at a greater distance than particles of glass or carbon. This difference may however be due to a difference in intensity of the stimuli.

Ameba reacts to dark spots in much the same way that it does to beams of light. The reactions are either positive, negative or indifferent. But they are negative in much the greater number of cases. But no sooner does one observe the reactions of an ameba to perpendicular beams of light and of darkness than the question arises as to the transfer of the stimulus to the ameba as well as the nature of it. How can an ameba sense a beam of light or darkness which never comes nearer to it than 100 or 150 microns? It is possible that small particles suspended in the water reflect light from a beam of light so as to reach the ameba in much the same way that man can observe a beam of light in a dark room because of the dust particles in the air. But if so the ameba, being eyeless, is wonderfully sensitive to light. But as to beams of darkness the case is entirely different. Is it conceivable that an ameba can sense a beam of darkness at a distance because not as much light is reflected from the particles in the dark beam as from those more brightly illuminated surrounding the beam? If one did not know of reactions to beams of darkness, one might adopt the hypothesis of the reflection of light from particles in the beam; but since similar behavior is observed toward beams of darkness, this explanation is obviously not the right one. Some disturbance is created by the beams which is then radially transmitted; so much is certain. But just what is the nature of the disturbance is not clear.

In a preceding paper (Schaeffer, '16*c*), in which the reactions of ameba to particles of glass, carbon, and similar materials were described, it was concluded that the nature of the stimulus which enabled amebas to react to these substances at a distance also remains unrecognized. Now it is possible that the nature of the stimulus which makes reaction at a distance possible is the same for all these various test objects, since the reactions are very similar. If so, the nature of the stimulation must be simple and fundamental, such as differences in electrical potential which give rise to electrical currents. But if the nature of the stimulation should be electrical, the quantities of current arising from the various test objects must be infinitesimally small, and very great if not insurmountable difficulties would be encountered in demonstrating the presence of such small currents.

To show the general reactions of ameba to globulin, carbon, etc., when stimulated simultaneously by beams of light or of darkness, the experiments may be classified as follows.

1. Food objects (grains of globulin) were laid *over* a beam of intense light so that the food should be very brightly illuminated —365–372. Blue spectral light was used in the experiments recorded, for blue light has been regarded as more disagreeable than other spectral colors. The globulin was sensed at a distance and the ameba moved toward it and ingested it. There was no definite indication that the blue light had any effect in modifying the behavior unless the pseudopod to the right in Fig. 368 is to be regarded as expressing a deterrent effect of the light. The ameba, in effect, reacted as if no spectral blue light was present.

2. The food substance was laid *some distance from* the green or yellow light, and in various positions with respect to the ameba and the beam of light—373–437.

(*a*) When the green light lay between the ameba and the globulin, the light had a slight disturbing and deterring effect —386. The ameba made a slight detour around the green light. In another test with the experiment similarly staged, the disturbing effect of the green light was more pronounced—373. The ameba made a wide detour around the light and moved into contact with the globulin without coming into contact with the green beam. In both experiments green light, which is positive when sensed alone, became negative in contrast with the more strongly (or differently) positive globulin.

(*b*) In the experiment with yellow light—397–407—the ameba moved straight toward the light after the globulin was within sensing range, then moved over the beam of light, after which the direction of motion was changed so that the ameba moved directly toward the globulin. The globulin was eaten in a typical food cup. The yellow light was not deterrent in this case. But another ameba reacted negatively to both yellow light and globulin, when presented simultaneously, but positively when presented separately. The ameba was satiated or sick, for the globulin was only partially surrounded.

3. Grains of globulin and carbon were laid over beams of darkness.

(*a*) An ameba moved toward a dark spot on which lay a grain of carbon until it came within about thirty microns of the dark spot, when negative behavior set in. The ameba moved away to the right—463. In the succeeding test the ameba reacted at first positively to the dark spot alone, and after that decidedly negatively.

(*b*) A piece of globulin was laid on the dark spot, to the right of the ameba. The ameba moved directly toward the dark spot—globulin—though it seemed to have been slightly deterred by the dark area, for the ameba broke up into two pseudopods—495—and just when the dark beam was reached a little later, a small side pseudopod appeared. The globulin was however finally ingested. In another experiment the globulin was placed near the far edge of the dark spot—522. The behavior of the ameba became very irregular as it moved near the dark beam. Soon a pseudopod was sent out straight toward the globulin, but it was presently retracted and the ameba moved off to the left, veering to the right. There can be no doubt of the strongly deterrent effect of the dark beams. There can also be no doubt of the strongly attractive effect of the globulin.

SUMMARY.

1. Ameba senses beams of light of twenty microns' diameter that pass no nearer to the ameba than 100 microns or 150 microns. In nearly all cases under these conditions the ameba moves directly toward the beam. When the ameba comes into contact

with the beam it either flows over it indifferently, or it reacts negatively to the beam by changing its direction of movement.

2. Beams of spectral light and of white light have approximately the same general effect. It appears however that spectral light at the blue end is somewhat less attractive than that at the red end.

3. Beams of darkness are also sensed at a distance like beams of light. They are usually negative. That is, the ameba usually avoids the beams before coming very near them.

4. It is the change of light intensity that determines changes in reactions. Neither high nor low intensities seem to be either negative or positive in themselves. Movement from a region of low light intensity into a region of high intensity frequently occurs if the contrast is not too great; but movement toward a region of lower light intensity (dark beams) is seldom seen.

5. No explanation is suggested for the sensing of beams of light and of darkness at a distance. The nature of the stimulus and the means of its transfer in such cases is not known.

6. Grains of globulin illuminated by perpendicular beams of light seem, on the whole, to be at least as attractive as when not more brightly illuminated than the field. But when globulin grains are laid in large dark beams, the ameba frequently shows unmistakable signs of a tendency to react negatively.

7. Both light beams and globulin grains are positive when stimulating the ameba separately; but when a grain of globulin and a beam of light, placed a small distance apart from each other, stimulate the ameba simultaneously, the more weakly positive object—the beam of light—becomes usually strikingly negative.

8. An ameba is positive, negative or indifferent to beams of light depending upon circumstances.

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Verworn, M.

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EXPLANATION OF PLATES.

The figures are camera lucida drawings of sample experiments taken from the laboratory notes without alterations. The camera lucida was attached to the right-hand tube of a long-arm Zeiss binocular microscope, which was used in connection with the stage and condenser of a compound microscope. Eyepieces number 4 and objective a_3 were used, giving a magnification of 65 diameters. A scale by means of which the size of amebas and light beams can be estimated is shown on Plate V.

The figures are numbered serially from 1 on, for reference. The numbers are placed inside the figures. They are to be looked upon as labels only. They have no other significance. An x following a number, as 21x, indicates the end of the experiment illustrated by Figs. 10 to 21x inclusive. A new experiment starts with Fig. 22 and ends with Fig. 36xx, and so on. If a number is followed by xx, it means that the next experiment was performed upon a different ameba. Thus Figs. 1 to 9xx represent the result of a single experiment upon an ameba. With Fig. 10 a new ameba was employed, and so on. The order in which the figures were drawn is represented by the serial numbers for all the figures in any one experiment, and in nearly every case for all the experiments performed on any one ameba.

The time of the beginning and the end of each experiment is given in hours and minutes. In some cases the time of drawing of each figure is also given, and where it is not given it may easily be computed—the figures in such case being spaced equally in time.

The arrows show the direction of active protoplasmic streaming. The larger arrow in the last figure of each experiment denotes the direction the ameba took in moving away from the test object.

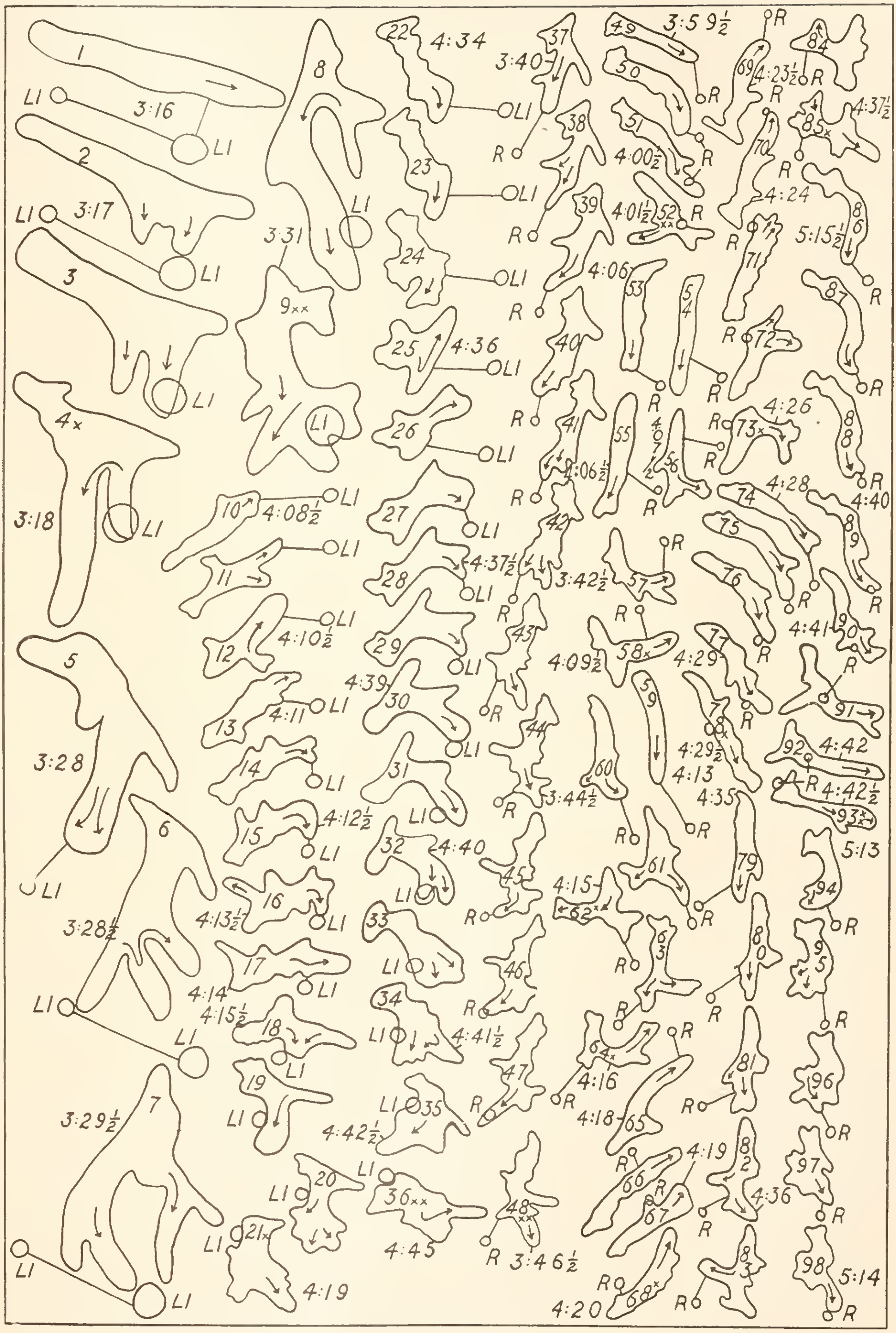
The light beams, etc., are labelled in abbreviated form. See table of abbreviations on below. For quick and correct reference these test objects are connected with the proper ameba by leader lines. These lines have no other significance.

It will be noted that there are slight differences in the size and shape of the same light beam or other test object as drawn in the figures of any single experiment. The explanation for this difference lies in the speed with which the drawings had to be made in order to catch important items of behavior. As a rule the parts of the ameba lying nearest the test object received the most careful attention and were drawn first; the posterior parts of the ameba and the test object were drawn last.

For detailed explanation of figures see pages 49–67 of the text.

TABLE OF ABBREVIATIONS.

B, blue light.
CA, carbon.
D, dark beams.
G, globulin.
GN, green light.
LI, white light.
OL, orange light.
R, red light.
V, violet light.
Y, yellow light.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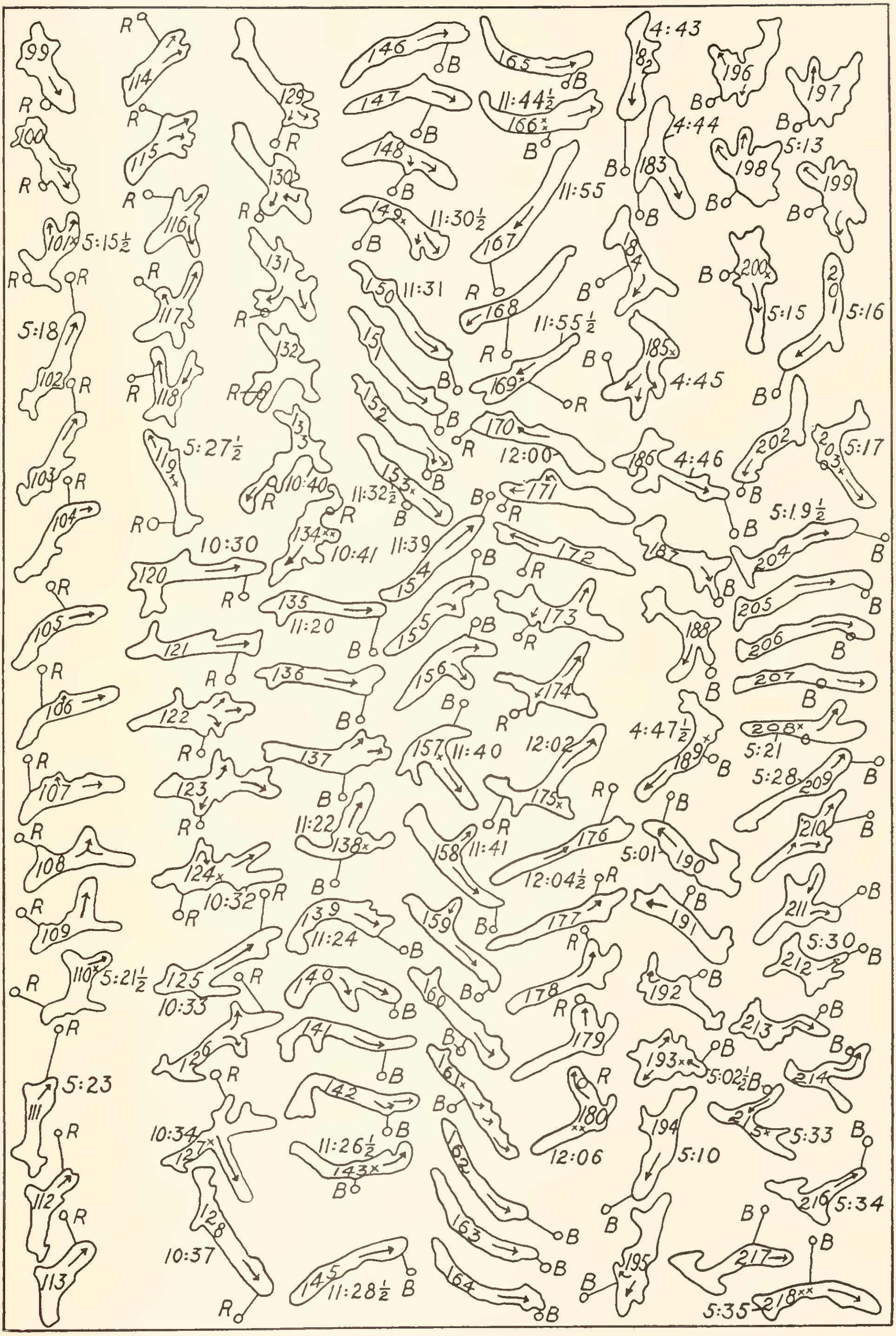
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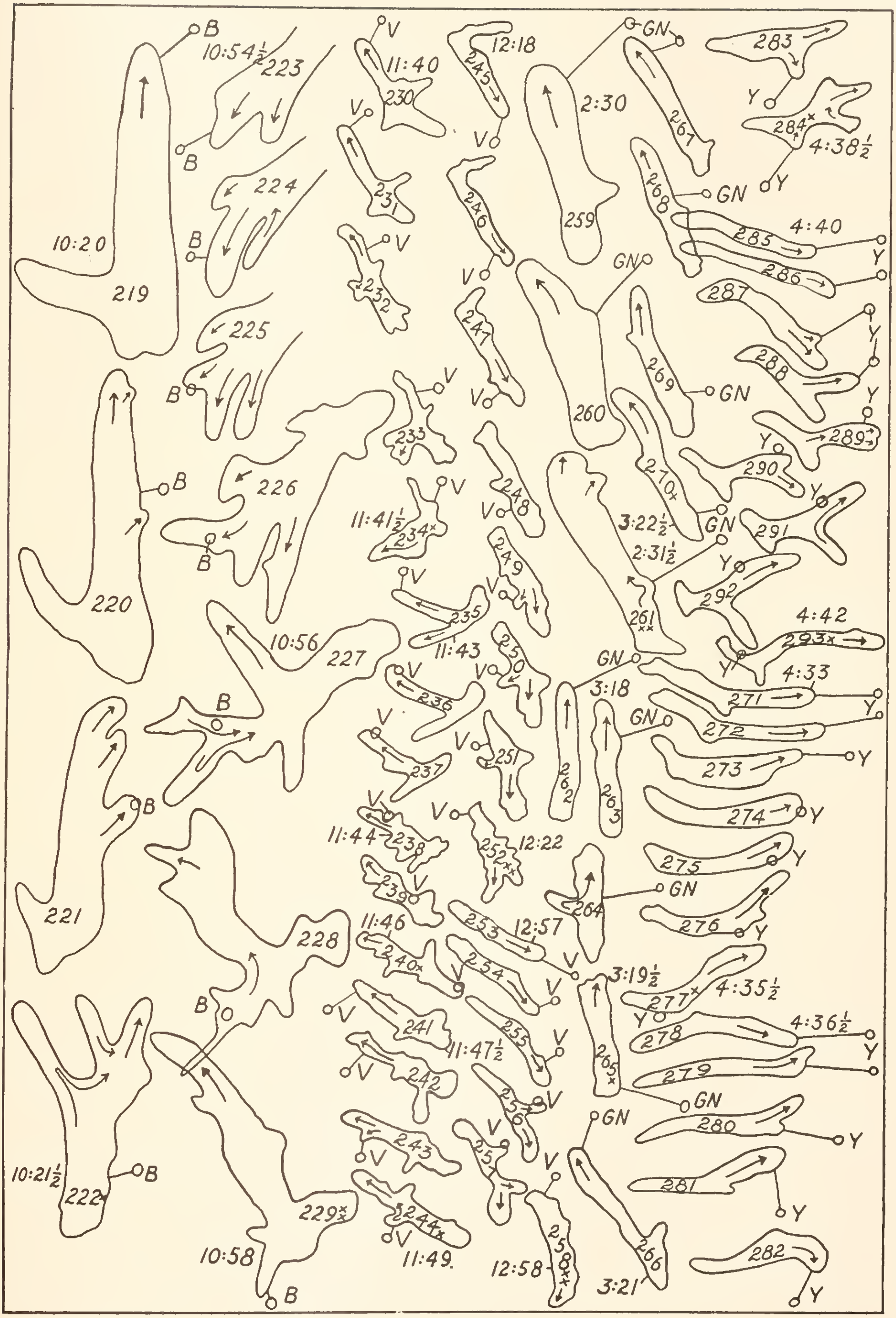
ASA A. SCHAEFFER.



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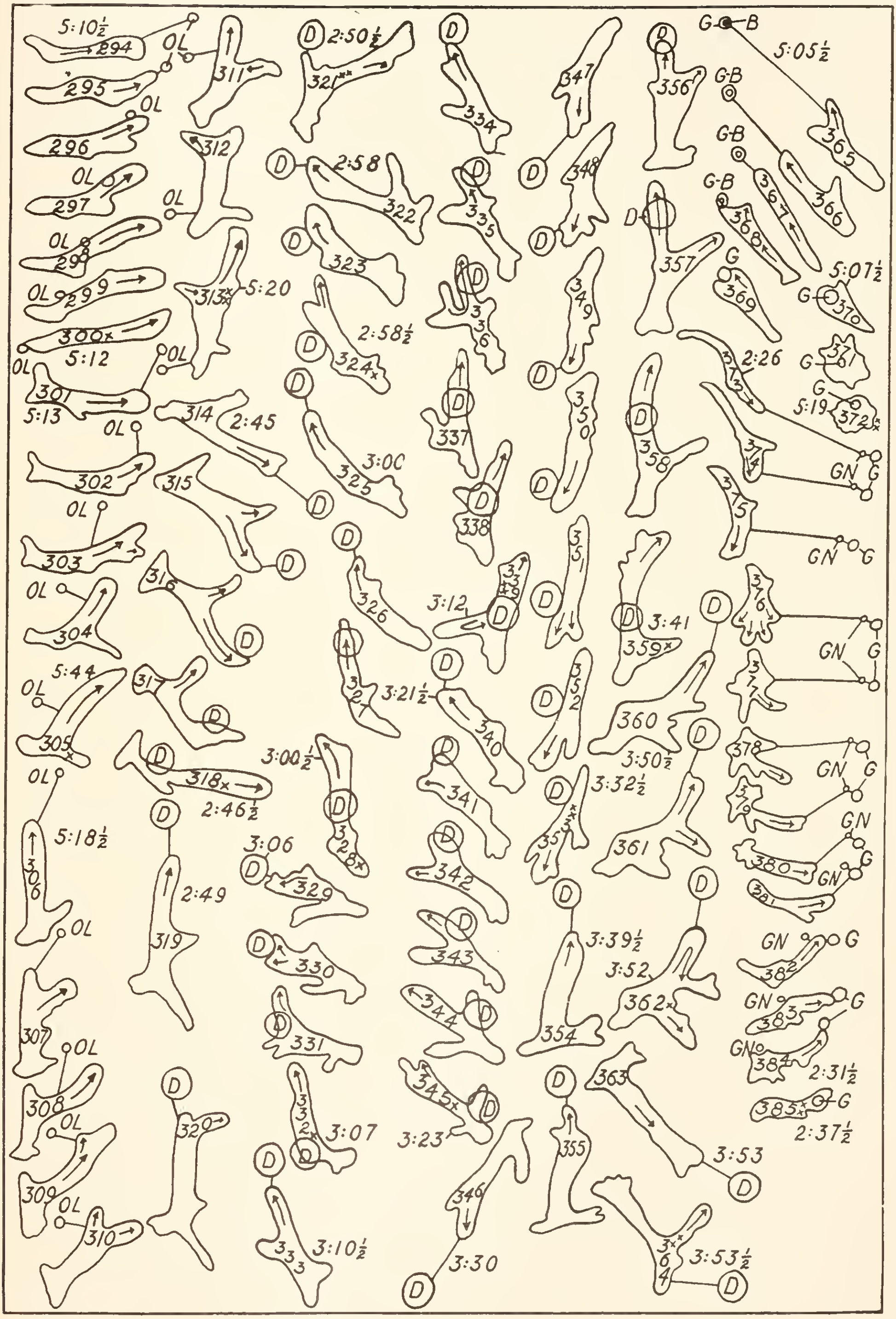
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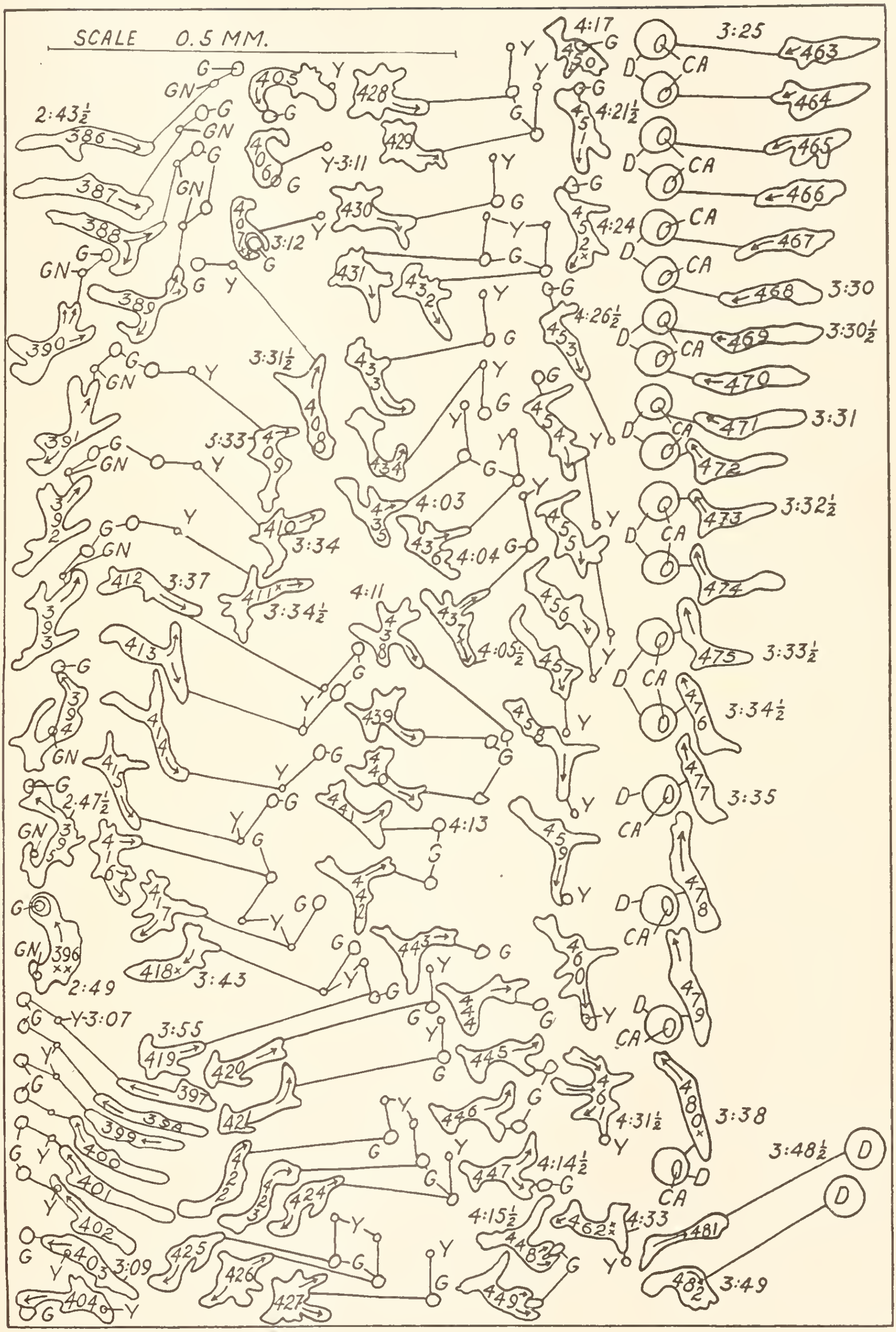
PLATE V.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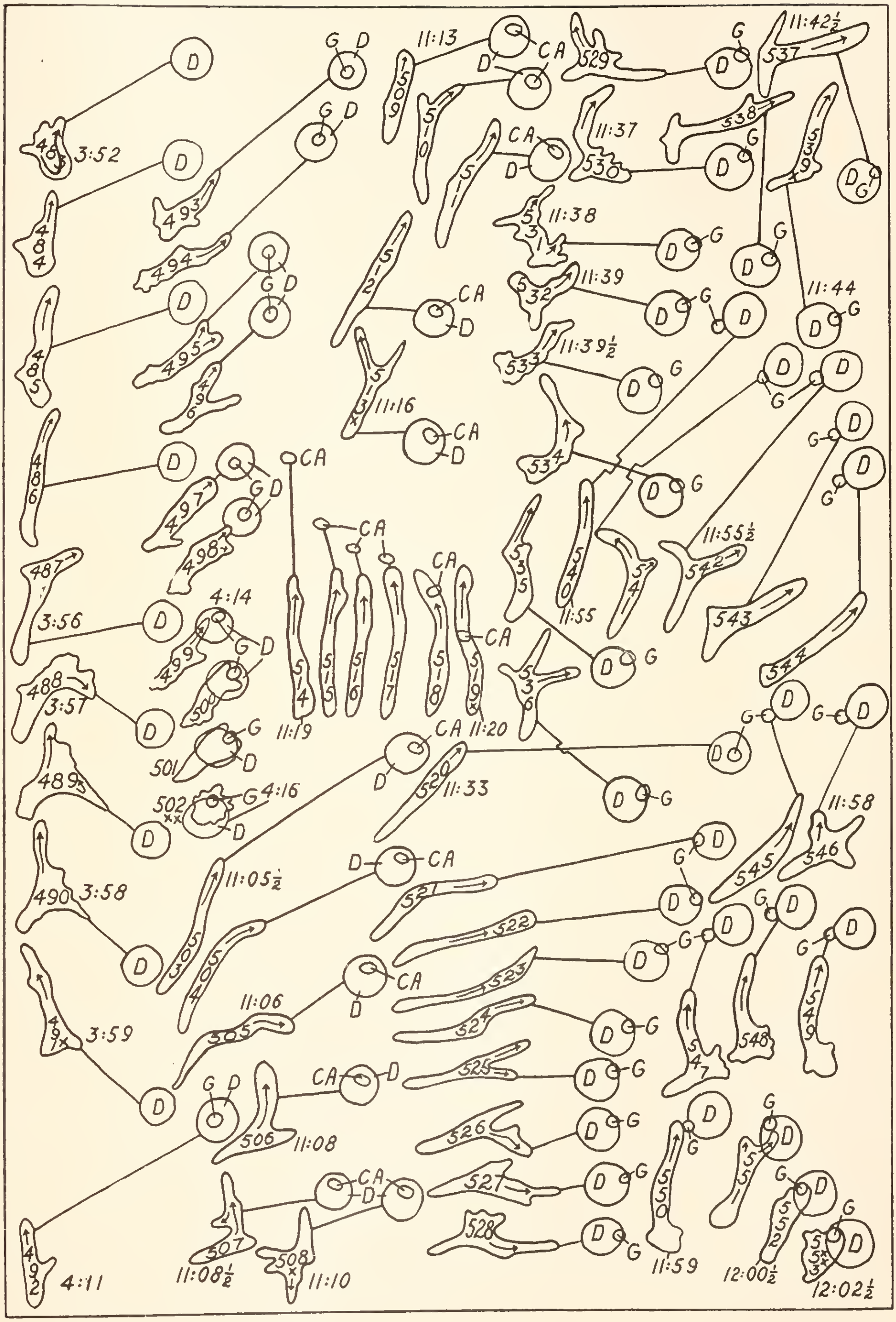
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ASA A. SCHAEFFER.

SUSCEPTIBILITY GRADIENTS IN THE HAIRS OF CERTAIN MARINE ALGÆ.

C. M. CHILD,

WITH FIVE FIGURES.

The colorless hairs or capillary branches so common on the red and the brown algæ are in many cases beautiful objects for the study of the axial susceptibility gradients. A few data obtained during the summer of 1915 at Woods Hole have already been published (Child, '16a). The present paper records the result of further investigation during the summer of 1916 on the physiological polarity of these hairs and includes data on additional species, experimental results on modification of the gradients which answer certain questions raised by the earlier work, and also a correction of the earlier observations concerning one species.

Thus far hairs of the following three types have been examined:

Unbranched unicellular hairs, *Ceramium*, *Chondrus*, *Agardhiella*. The hair is a very slender and delicate outgrowth, sometimes reaching a length of several millimeters, but unicellular.

Unbranched multicellular hairs with basal vegetative tip, *Fucus*, *Castagnea*.

Branched, multicellular hairs with apical vegetative tip, *Chondria*, *Polysiphonia*, *Griffithsia*.

Although this grouping of species according to the form of the hairs does not represent the taxonomic order, it seems the most satisfactory for present purposes since only the hairs are to be considered. In most species examined the hairs are so extremely sensitive that conclusions concerning the existence of a gradient and its direction can be safely drawn only from plants in the best possible physiological condition, collected with the minimum of handling and examined at most within a few hours after collection. Within the first few days in the laboratory, even in running water, the hairs usually die and often drop off, although other parts of the plant may remain alive and apparently in good condition.

In the earlier observations on the hairs (Child, '16) the extreme sensitiveness and rapidity with which alterations in the gradients may occur was not fully recognized, consequently some of the statements made at that time require correction.

Since the hairs are without pigment and their protoplasm is in most cases almost entirely devoid of visible structure, except under high powers, in the living condition a susceptibility gradient can be made visible only by staining with neutral red or some other vital dye and then killing with the proper concentration of some reagent. As killing agents neutral red, KCN and $HgCl_2$ were chiefly used.

Unbranched Unicellular Hairs.

Ceramium rubrum constituted the chief material for the study of hairs of this kind. In the earlier paper it was stated that "in the long full-grown hairs the susceptibility gradient is distinctly acropetal while in those which are apparently still growing it is basipetal" (Child, '16, p. 104). The later observations indicate that the normal or primary gradient is basipetal in all hairs, but that it may readily undergo reversal. In the observations of 1915 the hairs were first stained with neutral red and then killed in KCN *m*/100. In 1916 it was found that when neutral red was used both as staining and killing agent the more susceptible hairs which died during the first 1 to 2 hours showed almost without exception a basipetal gradient, *i. e.*, death began apically and proceeded basipetally, while among many less susceptible hairs which died later basipetal gradients were much less frequent, many hairs showed no definite death gradient, and acropetal gradients were often observed. Apparently if the neutral red kills the hair rapidly enough the death gradient is basipetal, but where death occurs only after a longer time the original gradient may be obliterated or even reversed. In short the neutral red may obliterate or reverse the original gradient in susceptibility to itself.

Hairs stained for a few moments in neutral red and then killed in KCN usually show a basipetal gradient, but when stained for an hour or more and killed in KCN the frequency of basipetal gradients is lower and that of acropetal gradients higher, *i. e.*, the neutral red tends to reverse the susceptibility gradient at least to some other agents as well as to itself. Discussion of these and other cases of reversal is postponed to the final section.

Ceramium rubrum is in general highly resistant to depressing conditions and the hairs are much less sensitive than those of many other forms. It has been observed, however, that in plants kept in the laboratory for several days the hairs are usually less susceptible, and acropetal gradients are more frequent than in fresh specimens.

The visible death-changes in these hairs are very similar to those in other cells (Child, '16a, '16b). A slight basipetal staining gradient may or may not be visible. Before the aggregation and decoloration of the protoplasm the usual change in color to deeper red occurs, and this color change commonly shows a basipetal gradient where the death gradient is basipetal. Often death is preceded by plasmolysis which proceeds in the same direction as death and not infrequently a more or less regular gradient in length of the plasmolyzed portions appears, the most apical portion being shortest and the length increasing basipetally (Fig. 1). In other cases the protoplasm may separate into only two or three portions, and death may also occur without preceding plasmolysis. Instead of separating into distinct portions the protoplasm often undergoes a plasmolytic contraction, as a single mass, retracting slightly as a rule from the apical end and basally from one third to two thirds of the hair length. Both the gradient in length of plasmolyzed portions and the difference in apical and basal retraction indicate a graded difference in protoplasmic condition along the axis.

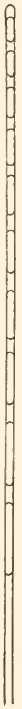

FIG. 1.

The final change in the protoplasm is the apparent collapse of the protoplast or its separate portions and the aggregation into small masses stained almost black. Where the basipetal gradient is well marked this change begins apically and can be followed basipetally along the hair. Where the gradient is obliterated it may begin at almost any level, or at several levels, or may be simultaneous throughout the length, and where the gradient is acropetal it begins at the basal end of the protoplasm and proceeds acropetally. The deep stain of the masses scattered along the cell rapidly fades and the hair presents almost the appearance of the normal hair, but a careful examination with proper illumination shows the aggregated protoplasmic masses which are still transparent and are visible only because of their refractive index.

The hairs of *Chondrus crispus* and *Agardhiella tenera* are similar in structure to those of *Ceramium* and the few incidental observations made on these species indicate that they behave in essentially the same way. The hairs on freshly collected plants in good condition usually show a basipetal gradient. Most of the hairs die during the first two or three days in the laboratory and acropetal gradients with decreased susceptibility are often observed before they die. Plasmolytic separation or basal retraction of the protoplasm also often precedes death and the final changes are similar to those in *Ceramium*. In the hairs of *Ceramium* and *Agardhiella* the basal retraction of protoplasm often occurs merely as the result of laboratory conditions and before death the entire protoplasm of the hairs may be contracted into a mass one fifth or less of the length of the hair, lying near the apical end and as might be expected, staining much more deeply than in the normal condition. Similar changes probably occur in the hairs of *Chondrus* but have not as yet been looked for.

Unbranched Multicellular Hairs.

Hairs of this kind consist of a single series of cells (Figs. 2 and 3), and in the only two species examined, *Fucus vesiculosus* and *Castagnea tuberculosa*, the vegetative point of the hair is at its attached basal instead of at the free apical end. Because of this fact observations on the susceptibility gradients of these hairs are of particular interest.

In *Fucus* the hairs develop from the inner walls of flask-shaped cavities on the thallus, and later from the walls of the conceptacles. The hairs reach the exterior through the narrow neck of the cavity and form dense tufts, often several millimeters long. As noted above, the growing point of the hair is basal, *i. e.*, next to the wall of the cavity and is therefore not visible in intact specimens, but the fact that the length of the cells increases from the most basal region visible toward the free end suggests that cell division is basal instead of apical. Fig. 2 shows a hair of *Fucus*, the basal concealed portion being diagrammatically indicated in dotted lines.

In freshly collected plants the hairs are extremely sensitive and even in dilute neutral red death begins within a minute or two, often before the preparation can be placed under the microscope, and in a few minutes the whole hair is dead. In such hairs the gradient is acropetal, *i. e.*, the basal region of the hair is the region of highest susceptibility. The acropetal gradient is very distinct and uniform in the progress of death from cell to cell and in the longer, older cells nearer the free end the acropetal intracellular gradient can usually be seen if death is not too rapid. In the shorter, younger cells an intracellular gradient is usually not visible in the concentrations ordinarily used for killing. The high susceptibility of the hairs makes it impossible to stain with neutral red and then use other agents for killing.

At the time of my first observations on the susceptibility of these hairs I was entirely ignorant of the fact that they grew from a basal instead of an apical vegetative point or "growing tip," but the presence of very distinct acropetal instead of basipetal gradients in all hairs of freshly collected plants constituted practically conclusive evidence for the existence of a basal vegetative region and examination of the cellular structure of the hair and reference to the literature confirmed the conclusion. In this case then the direction of the gradient made possible the immediate recognition of the rather unusual growth form.

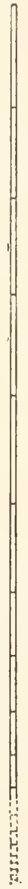

FIG. 2.

In freshly collected plants in good condition and very carefully handled, particularly in small young thalli, there may be practically no irregularities or exceptions to the regular acropetal course of death in the hairs of the whole thallus. After a few hours in the laboratory, however, or after exposure to unfavorable conditions, *e. g.*, partial drying at low tide, the change in the condition of these hairs is very striking.

The most susceptible basal region is almost invariably dead, the dead region usually including the two or three most basal cells visible, and the gradient is usually completely reversed in the more distal regions of the hair. Instead of being practically instantaneous, as in the hair when in good condition, staining with neutral red occurs very slowly, and a basipetal staining gradient appears, *i. e.*, the most distal cell stains most rapidly and the rate decreases basipetally from cell to cell. The same basipetal gradient appears in the course of death.

In these hairs with reversed gradient the susceptibility of the most distal region is not much altered; this is the least susceptible region, the last to die in the normal hair and the most susceptible in the hair with reversed gradient, but its susceptibility is very much the same in the two cases. The more basal the level, *i. e.*, the higher the original susceptibility, the greater the decrease in susceptibility afterward, until in the more basal regions the cells which were originally most susceptible of all are dead.

The cells next to these dead basal cells show the structure of dying cells as soon as they begin to stain, *i. e.*, masses of aggregated protoplasm which stain almost black are already present, but include only a part of the protoplasm, and the remainder stains diffusely in the usual way. When the death of such cells is finally completed the remainder of the protoplasm is aggregated into deeply staining masses in the usual way and the diffuse stain disappears. In these cases the death-changes in a single cell may extend over hours. It is a fact of considerable interest that the cellular death-changes resulting from unfavorable conditions in the laboratory or in nature are the same in appearance as those occurring in neutral red and other killing agents.

The very great change in susceptibility in some cells of the hair after reversal of the gradient is indicated by the fact that the cells of the more basal levels which die in one or two minutes in neutral red when the hair is in good condition may survive for twenty-four hours in neutral red after reversal.

What happens in such reversal is apparently this. The hair is very sensitive and under depressing environmental conditions the basal, most susceptible region dies as it does in solutions of killing agents in the laboratory. Next to this region are cells, originally somewhat less suceptible, and these are "almost" or "partly" killed and their metabolic rate so decreased and protoplasmic condition so altered that they become very insusceptible in killing agents. Still farther distally the effect of the altered conditions is less marked until finally little or no change occurs in the most distal cell. If we now subject a hair altered in this way to the action of a killing agent such as neutral red, KCN, etc., we find that the altered condition appears in the altered susceptibility to the killing agent. The fact that the changes produced by some external agent or complex appear as altered or reversed susceptibility to another is of interest and brings up the question whether the action of the two agents is additive or not. But whatever the nature of the processes, the fact of reversal is sufficiently evident, and, as in other cases, supplements the direct evidence for the existence of a dynamic gradient.

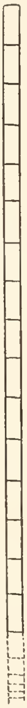

FIG. 3.

Castagnea tuberculosa bears very fragile unbranched multicellular hairs, which like those of *Fucus* develop from a basal vegetative point concealed below the surface of the thallus. Fig. 3 shows the general appearance of the hair, the concealed basal portion being diagrammatically indicated in dotted lines. In my earlier paper (Child, '16*a*, p. 101) I stated that the gradient in these hairs is in general basipetal, but with frequent irregularities, and the earlier observations of 1916 also showed in general a basipetal gradient. In all these cases, however, the plants used had been in the laboratory a day or more and further investigation on fresh material showed that the gradient is primarily acropetal, but very readily undergoes more or less complete reversal as in *Fucus*. In fact, even in freshly collected material apparently in good condition, but with long well-developed hairs, reversal has been observed in many cases.

At the time of the earlier observations I did not realize how readily reversal of the gradient might occur nor was I aware that the vegetative point in these hairs is basal and not apical, and when a well-marked basipetal gradient was found as a rule in plants which had been in the laboratory a day or two, I failed to realize the necessity of checking up the results on perfectly fresh material. The cells of the *Castagnea* hair attain their full size very near the basal end, so that in that portion of the hair visible in the living plant the cells are all of practically the same diameter and length and it is difficult or impossible to determine the position of the growing point from a gradation in cell size. This case shows how readily incorrect conclusions may be drawn where the material is not in perfect condition. These hairs, like those of *Fucus*, are very sensitive and the gradient undergoes reversal very readily, but there can be no doubt that it is primarily acropetal, not basipetal, as stated in my earlier paper. The hairs are so fragile that they are readily broken or injured mechanically and at least many of the irregularities are due to such injuries.

BRANCHED MULTICELLULAR HAIRS.

Among hairs of this kind those of *Chondria* have served as the chief material, but the hairs of *Polysiphonia* and *Griffithsia* have also been examined. The hair of *Chondria* develops by repeated apical dichotomy (Fig. 4), and since growth is primarily apical a basipetal death gradient is to be expected. In 1915 hairs of this species were examined by first staining deeply with neutral red and then killing in KCN and the gradient from cell to cell was found to be distinctly basipetal while within single cells an acropetal gradient was almost invariably observed (Child' 16*a*, pp. 105–106). Since these observations were made during the last days of my stay at Woods Hole in 1915 there was no opportunity to repeat the observations with fresh material until the following year. As regards the acropetal cell gradient the results of 1916 are as follows: When the hairs are killed in a high concentration of neutral red alone (*e. g.*, before precipitation in sea-water) both hair gradient and intracellular gradient are very uniformly basipetal. Similarly when the hairs are killed in some other agent (KCN $m/1000 - m/100$, $HgCl_2$ $m/50000$) to which a few drops of neutral red have been added to stain the hairs slightly and so make the death-changes clearly visible, both hair-gradient and cell-gradient are basipetal. On the other hand, when the hairs are first deeply stained with neutral red and then killed in KCN or some other agent, the method used in 1915, the hair gradient is basipetal but the cell gradient is very generally acropetal.

Evidently the acropetal cell-gradient observed in 1915 is not a natural condition but represents a reversal of the cell gradient by neutral red or by other conditions, and this appears as a reversed susceptibility gradient to KCN or $HgCl_2$. These hairs take up neutral red rapidly and stain very deeply, and the axial differences in susceptibility to neutral red within the elongated cell are sufficient to bring about reversal of the susceptibility gradient after a time. If death occurs early the reversal does not appear, but if the concentration of neutral red is low enough to allow survival for two or three hours in the solution, reversals of the cell gradient begin to appear.

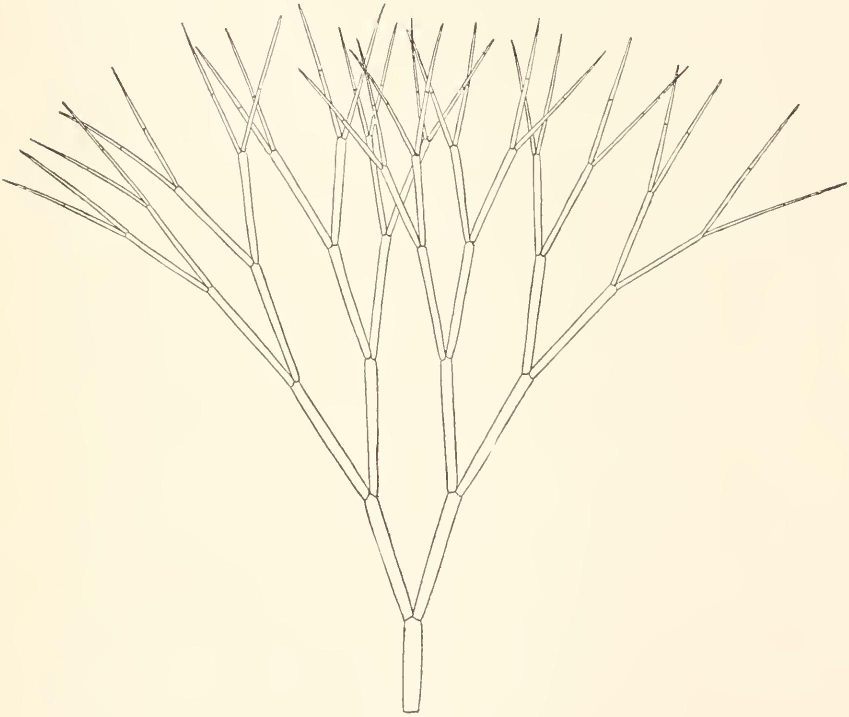

FIG. 4.

Further observations showed that after two or three days in the laboratory the intracellular gradient was frequently reversed, particularly in the more basal cells of the older hairs. Similarly in plants found detached reversed intracellular gradients were often observed. Such plants torn loose by the waves and washed into shallow water may be exposed to depressing conditions of temperature, light, or partial drying at low tide. There is of course no way of determining what or how extreme these conditions have been in a particular case but the fact that reversed intracellular gradients appear frequently in the hairs of such plants but are not present in material which so far as can be determined is in the best physiological condition, together with the data on experimental reversal, indicate clearly enough that such reversals are the result of depressing or toxic factors.

Whatever the processes involved in such reversals, they, like other reversals described in this and an earlier paper (Child '16b), are dependent on the preëxistence of a basipetal susceptibility gradient in the cell, and they represent the differential action of external factors within certain limits of concentration or intensity upon this gradient. The difference in susceptibility between different cells, even two adjoining cells, is usually greater than the differences between the two ends of a single cell, and the difference between apical and basal cells of a hair is of course very much greater than any intracellular differences. The persistence of the hair gradient with reversal of the intracellular gradient indicates merely that the relations between the lesser differences in susceptibility are more readily reversed than those between the greater differences.

The establishment of the fact that the acropetal intracellular gradient is a reversal of the original gradient at once raises the question whether the gradient of the hair as a whole can be reversed. Various attempts at such reversal have been made with some success, particularly in the younger hairs, but in the full-grown hairs the axial differences in suceptibility are so great that complete reversal has not as yet been induced and is perhaps impossible.

In *Chondria* the hairs occur in large numbers near the apical ends of branches of the thallus, and in cases where extensive hair-development occurs the hairs appear macroscopically as white tufts covering the branch tips. A well-developed tuft of this kind is made up of hairs of very different stages of development and physiological age, the most basal hairs being in general the earliest developed and so the largest and the oldest, while the most apical are the latest, smallest and youngest, with intermediate stages between the two extremes. The differences in physiological age in such a group are beautifully shown by the differences in susceptibility, the youngest hairs being most susceptible, the oldest least, with a general gradation in susceptibility between. These age differences appear even in the susceptibility of the apical cells, those cells of the young hair being somewhat more susceptible than those of the old, but this difference between apical cells of young and old hairs is much less than that between the more basal cells. In KCN *m*/1000 for example, apical cells of the youngest hairs die almost at once, those of the oldest hairs after 1–5 minutes, but in the youngest hairs death is complete all the way to the basal cell in 10–15 minutes while in the oldest the most basal cells may live for 2–3 hours. In the young hair the basal cells have not yet had time to become old, while in the old hair the basal cells have reached an advanced stage of physiological senescence, and in the apical cells senescence has progressed to a greater or less extent, but has been counterbalanced to some extent by the periodic rejuvenescence accompanying cell reproduction (Child, '15*a*, Chap. X.). These susceptibility relations between the older and younger hairs of a single group and between apical and basal regions of a single hair constitute a most beautiful and striking example of the course of senescence in plant axes.

The attempts to reverse the whole hair gradient were made with tufts of this sort, consisting often of hundreds of hairs of all ages and stages of development, their attachment to the branch from which they arose being of course undisturbed. In this way it was possible to observe the difference in the reaction of old and young hairs to particular experimental conditions.

The result of a few experiments along this line which serve merely to suggest the possibilities, are briefly stated. Exposure to temperatures of 30°–35° C. for three or four hours killed the youngest hairs completely or all but one or two basal cells, almost without exception. The hairs of medium age and susceptibility, however, showed a high percentage of complete reversals of the gradient, death beginning basally and proceeding apically, the apical cell being the last to die. In some other hairs of this group partial reversal of the hair gradient occurred, *i. e.*, the apical cell died first, and one or two cells below it in basipetal order, while in more basal regions the progress of death was acropetal, both from cell to cell and within the cells. In the oldest hairs the hair gradient was usually basipetal, in the apical third or half of the hair, *i. e.*, for a length of three to five cells, although the intracellular gradients were often acropetal, particularly in the more basal cells of this region. In the basal half or two thirds of such hairs there was usually more or less reversal of the hair gradient, the basal cell dying first, and one, two or even three cells apical to it dying in acropetal order. The intracellular gradients were very commonly acropetal in this region. In short, by this exposure to high temperature the most susceptible hairs were entirely killed, those of medium susceptibility showed complete or nearly complete reversal and the oldest showed more or less reversal in the basal regions. The susceptibility of all living hairs and cells was of course much lower than that of corresponding hairs or cells in good condition tested at the same temperature, and the susceptibility of the hairs of medium age in which the gradient had been reversed was usually as low as, or even lower than that of the oldest hairs. The experiment shows a gradient in injury by the high temperature both in the single hair and corresponding to age-differences in the tuft, the degree of injury varying in general with the normal susceptibility of different hairs and different levels.

With short periods of exposure complete reversal was obtained in the youngest hairs in many cases, partial basal reversal in those of medium age and in some of the oldest hairs, but usually only intracellular reversal in the more basal cells of the latter. In this case the susceptibility of the youngest hairs was often decreased below that of the oldest.

Staining in neutral red so dilute as to give a barely perceptible yellowish tinge to the water induced complete or nearly complete reversal in some of the youngest hairs, but only more or less reversal of intracellular gradients in the older. It was also found that in some plants the gradients of the youngest hairs were almost completely reversed after two or three days in the laboratory and after a day or two more these hairs were dead.

Of course intermediate conditions between the normal and reversed gradient are frequently observed. In such conditions the intracellular gradient may be absent or very irregular and the hair gradient may be slight or even absent over a length of several cells. The differences in susceptibility between apical and basal end are, however, so great, particularly in the older hairs, that at the stage when the gradient is obliterated in one region of the hair other portions are either still normal, already reversed or dead.

Where the gradient is obliterated or reversed the hairs show a marked tendency to separate into their individual cells. Apparently the persistence of the orderly multicellular axis is associated with the persistence of the gradient. Other cases in which this separation appears will be described later.

The hairs of *Polysiphonia* are similar in structure and growth to those of *Chondria*, but in *P. variegata* and *P. fibrillosa*, the only species examined thus far, they are considerably more susceptible than the *Chondria* hairs and therefore not so favorable for experimental work. The gradient is primarily basipetal both in the hair in general and in the cell, and while more or less reversal has sometimes been observed, experimental reversal has not been attempted.

The hairs of *Griffithsia* differ from those of *Chondria* chiefly in the whorled arrangement of branches (Fig. 5) and as we should expect, since growth is primarily apical, both general and intracellular gradients are basipetal when the hair is in good condition. After two or three days in the laboratory, however, irregularities and reversals appear, and the hairs soon separate into cells or fall off entire.

Discussion.

It is evident that at least the hairs of these three kinds among the algæ show axial susceptibility gradients like those in the vegetative axes of the thalli. Moreover, these gradients show a direct relation to the location of the vegetative point, the region of highest susceptibility being in general the region of the vegetative point, at least when the hair is in good condition.

In *Fucus* and *Castagnea*, for example, where the vegetative point is basal, the gradient is normally acropetal, while in all other forms examined the vegetative point is apical and the gradient normally basipetal.

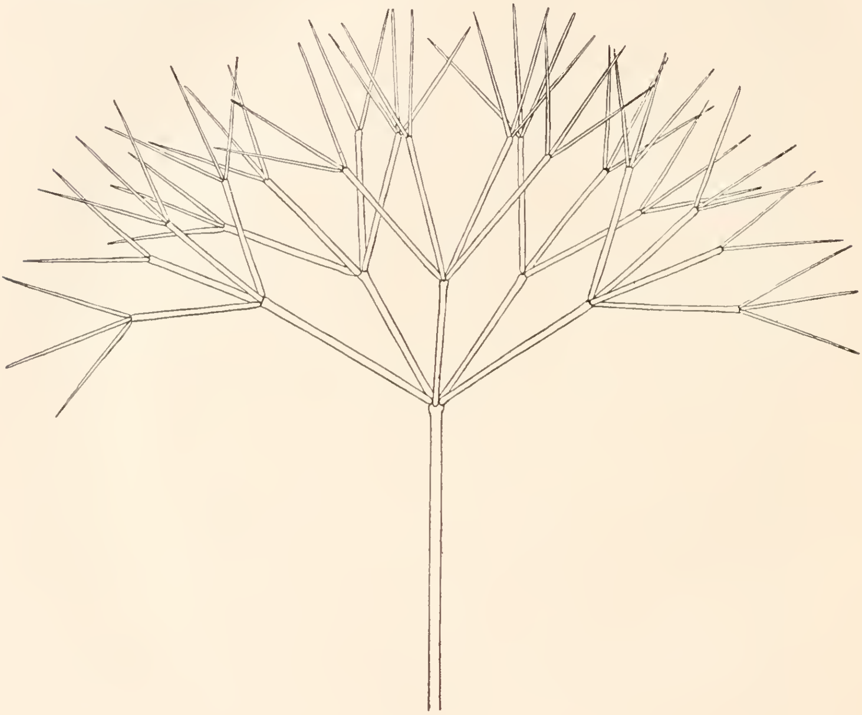

FIG. 5.

Under depressing conditions which are not extreme enough to kill rapidly the gradient may be more or less completely obliterated or even reversed, and such changes are of course a consequence of the differential susceptibility originally present. Obliteration or reversal of a gradient by external factors means simply that a region of highest susceptibility or metabolic rate, is affected by the external factor to so much greater extent than a region of lower susceptibility or rate that the original differences are obliterated or even reversed. In some cases an external agent may reverse the susceptibility to itself, as in the case of neutral red, and it is also possible in many cases to determine the effect on susceptibility of one agent by using another for killing after exposing to the first. The whole complex problem of the nature of the action of external agents of different kinds on protoplasm is involved in these data on susceptibility, but even though we are far from the solution of that problem we can establish certain general laws of susceptibility as characteristic features of a physiological axis, *i. e.*, as an expression of polarity.

When the gradient is obliterated or reversed multicellular hairs often separate more or less completely into individual cells. The persistence of the hair as a multicellular order seems to be associated with the existence of the gradient. It will be shown elsewhere that this is true, not only for these hairs, but for the vegetative axes of certain species of algæ. Tobler ('02, '04) has observed in various species of the red algæ this tendency to separate into cells under unfavorable conditions and has used it as a method for inducing experimental reproduction. In the isolated cells of the hairs reproduction can scarcely be expected, but the relation between morphological order and physiological correlation is evident. After obliteration or reversal of the dynamic axial gradient the morphological order soon ceases to exist except where the structural stability is high.

A question of some interest concerning what may be called the death-level arises particularly in connection with the *Chondria* hairs. The young highly susceptible hairs of *Chondria* die without perceptible reversal of the gradient under conditions which bring about complete reversal in the hairs of medium age and some reversal of intracellular gradients with perhaps partial reversal of the hair-gradient in the oldest hairs. To reverse the gradient before death in the youngest hairs less extreme conditions must be used. These and various other facts suggest the possibility that the death-point, or more properly speaking, the death-level is not necessarily the same quantitatively for more active and less active protoplasm of the same kind and for more and less rapid killing, in other words the death-level may be relative rather than absolute. With our present lack of knowledge this remains merely a possibility.

This possibility has already been suggested in connection with certain observations on susceptibility in the lower animals (Child, '13, pp. 118–119) and it seems worth while to call attention to it in this connection. Actually the death-level, whether relative or absolute, must be a resultant of various component factors such as aggregate condition of colloids, permeability, rate of metabolism or of certain reactions, etc., and there can be little doubt that different agents and conditions act primarily or chiefly on different factors in this complex. But since these are factors in determining the behavior of a system, *i. e.*, are correlated, not independent, changes in one cannot proceed beyond a certain limit without inducing changes in the others. This being the case, the death-level as a quantitative level at which persistence of the system as a whole becomes impossible, must be for each particular case of death a more or less definite level or region of the curve which represents the quantitative changes in the activity of living protoplasm or its component factors. On the other hand, the previous condition of the system and the character and rate of action of the killing agent may play a rôle in determining this level in different cases. If this suggestion is correct, then regions or cells of different susceptibility in the hairs do not necessarily attain exactly the same condition at the point of death, and the condition in a particular cell or region may differ according as it is killed rapidly or slowly.

Since the hairs represent real physiological axes, similar to other plant axes the question of their physiological relation to other axes must be raised. Unquestionably the development of a hair axis is a form of reproduction in a protoplasm specialized in some way. As a reproductive process it must result from physiological isolation (Child, '11, '15*a*, Chap. IX., '15*b*, Chap. V.) and, as I have pointed out, physiological isolation and reproduction may occur, not only as the result of increase in size but also under depressing conditions in consequence of decreased dominance, *i. e.*, decrease or obliteration of a preëxisting axial gradient. Hairs frequently appear on young apparently vigorous plants, but they are often very characteristic of advanced vegetative stages or of plants which seem to be in poor condition. In *Agardhiella*, for example, I have frequently observed that parts of the fronds bearing hairs show a lower susceptibility than other parts (Child, '16b). The hair is an axis arising from a cell of a previously existing axis. Like any other physiological axis it represents a metabolic gradient and originates as such a gradient in a cell which, though apparently specialized in certain respects is still capable of undergoing growth and division. In species where the hairs are not extremely sensitive to laboratory conditions it will probably be possible to control their occurrence and development by controlling the metabolic condition of the plant and in this way to learn something of their physiological relation as axes to the other axes of the plant.

SUMMARY.

1. The unbranched unicellular hairs of *Ceramium, Chondrus* and *Agardhiella*, the unbranched multicellular hairs of *Fucus* and *Castagnea* and the branched multicellular hairs of *Chondria*, *Polysiphonia* and *Griffithsia* all show an axial gradient in susceptibility to various agents.

2. In the hairs of *Fucus* and *Castagnea* in which the vegetative point is basal the normal hair gradient is acropetal while in the other species where growth is primarily apical the gradient is basipetal, *i. e.*, in all cases the region of highest susceptibility is the region of the "growing tip."

3. Obliteration or the reversal of the susceptibility gradients has been observed or induced in the hairs of *Ceramium*, *Fucus*, *Castagnea* and *Chondria* and to some extent in those of *Griffithsia*. These changes in the gradient are dependent on the differential susceptibility to various depressing agents and conditions, which is a feature of the normal gradient. In general a high concentration or intensity kills so rapidly that reversal of the gradient cannot be demonstrated, but with lower concentrations or intensities the original differences in susceptibility may be obliterated or reversed before death occurs. In some cases a particular agent may reverse the susceptibility gradient to itself, and in others a reversal of the gradient produced by one agent may be demonstrated by the differential susceptibility to another.

4. The hair, like the vegetative portions of the plants, represents a physiological axis and the susceptibility gradient is one

aspect of the difference in metabolic and protoplasmic condition along the axis, in other words of the physiological polarity. The multicellular hairs often tend to separate into their cells when the gradient is obliterated or reversed, and the loss of the hairs under laboratory conditions or in other unfavorable environment is associated with the change in the gradient which usually occurs under such conditions.

Hull Zoölogical Laboratory,
University of Chicago,
November, 1916.

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THE SALT CONTENT OF NATURAL WATERS IN RELATION TO RHEOTAXIS IN ASELLUS.[1]

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Much of my work on rheotaxis of the isopod *Asellus communis* Say has been based on the fact that pond and stream mores of the same species give different rheotactic reactions. Among other differences the pond mores have a low, and the stream mores, a high degree of positiveness ('12). I have wished for some time to determine whether there were differences in the salt content of the two classes of habitats that might account for the differences in behavior. Upon my return to the Chicago area after an absence of three years, I hastened to make this survey. The subject is the more interesting since experiments in the meantime have shown that the rheotactic reaction may be controlled by various salts when present in sufficient concentration.

Through the kindness of Mariner and Hoskins, to whom I was already indebted for similar analyses, the spring and autumn salt content was determined for County Line Creek, near Braeside, Ill., and for a point near Osborn, Indiana (Slough 93; Shelford, '13). Many of the isopods used in experimental work have been obtained from these habitats. The analyses are from approximately maximum-minimum water level for the year 1916 and are given in detail in Table I.

Of the elements found, calcium and magnesium are known to decrease the positiveness of rheotaxis in *Asellus* when present in sufficient concentration. If the salt content is at all responsible for the low positive rheotactic response of pond isopods, its effect should be most marked in the most concentrated water analyzed. This water contained 228.4 mg. of calcium per liter of water, the

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[1] I am indebted to the Elizabeth Thompson Fund and to the Bache Fund of the National Academy for money grants and to Mariner and Hoskins for water analyses without charge; without their aid this work could not have been done.

Table I.

Showing Maximum-Minimum Water Level Analyses of Water from County Line Creek, Braeside, Ill., and Slough 93, Osborn, Ind.

The solids are shown in parts per million by Mariner and Hoskin's analyses and the gases in c.c. per liter.

	Stream.		Pond.	
	Maximum.	Minimum.	Maximum.	Minimum.
Nitrogen as free ammonia	0.024	0.023	0.320	0.160
Nitrogen as albuminoid ammonia	0.150	0.098	0.160	0.288
Nitrogen as nitrates	None	0.700	0.840	0.800
Nitrogen as nitrites	0.0015	0.002	0.0012	None
Chlorine	5.000	12.000	72.000	277.000
Iron	0.200	1.400	0.500	1.400
Calcium	50.300	61.850	72.500	228.400
Magnesium	0.360	33.700	0.120	45.300
Sodium	45.2	8.0	45.200	116.700
Potassium	2.700	Trace	6.900	Trace
Free oxygen	6.8	3.0	5.1	1.6
Free carbon dioxide	3.2	2.5	8.6	11.7
Half bound carbon dioxide	79.5	82.6	113.4	119.6

equivalent of the amount of calcium contained in 0.011 normal solution of calcium chloride. Supposing for the moment this calcium were the only cation present in the water it would have no depressing action on rheotaxis.

Witness:

Isopod 146 gave a rheotactic reaction of 30 per cent. +, 50 per cent. −, 24 per cent. α when first tested and after being in 0.05 normal solution of calcium chloride for five days the rheotactic response was 90 per cent. +, 10 per cent. −.

Isopod 147 when first tested gave 60 per cent. +, 10 per cent. −, 30 per cent. α and after 48 hours in a similar solution of calcium chloride gave 40 per cent. +, 30 per cent. α, 30 per cent. 0.

Three other isopods (Nos. 175, 176, 177) after 10 days in the same strength calcium chloride solution gave an average response of 37 per cent. +, 30 per cent. α, 33 per cent. 0 while the average rheotactic reaction of 253 untreated isopods from similar environmental conditions was 31 per cent. +, 45 per cent. −, 22 per cent. α, 2 per cent. 0.

But calcium is not the only cation present. Magnesium, a less powerful isopod depressant than calcium, is found to the extent of 0.0037 normal solution. If at this strength magnesium retains its depressing power, which laboratory tests similar to those just cited renders improbable, its action would be offset by the sodium which has an antagonistic effect and which is present in sufficient quantity to neutralize any possible depressing action of the nagnesium and to antagonize partially the action of the calcium, if that were needed.

Theoretically then, from the analyses of salt content, one would not expect the pond water to be less favorable for positive rheotactic reaction than the water from the stream; and this expectation was verified by laboratory tests. Stream isopods were kept in the laboratory for three days in water brought from their stream habitat. Then they were divided and part were put into pond water from the Osborn, Indiana, pond. Both lots of isopods were kept under identical conditions of temperature, aeration, and food supply for twenty days, during which time the eighteen tests shown in Fig. 1 were made. The graphs there show the percentage of positive reactions given daily by groups of five isopods chosen at random from the stocks under observation. The variation in positiveness is due in part to this chance selection of individuals for the daily tests ('13*a*) and in part to the setting in of an abnormal breeding season, brought on by the transfer from freezing stream water to a laboratory of about 15 degrees centigrade. The decline of positiveness is the usual effect of the breeding season.

For the eighteen days when comparative tests were made the stream isopods in pond water averaged 73 per cent. positive while their mates in stream water were 70 per cent. positive. This means that the difference in salt content of the two waters did not affect rheotaxis.

The most obvious difference in the two environments is the difference in the oxygen and carbon dioxide tension in the water. In the more extended work of five years ago, the oxygen tension of County Line Creek was found to lie normally between 5 and 10 c.c. per liter and the carbon dioxide tension was about 2 c.c. per liter. In the ponds the oxygen was usually under 3 c.c. per liter and the free carbon dioxide usually over 10 c.c. per liter. The amount of half bound carbon dioxide is also consistently greater in the pond than in the stream.

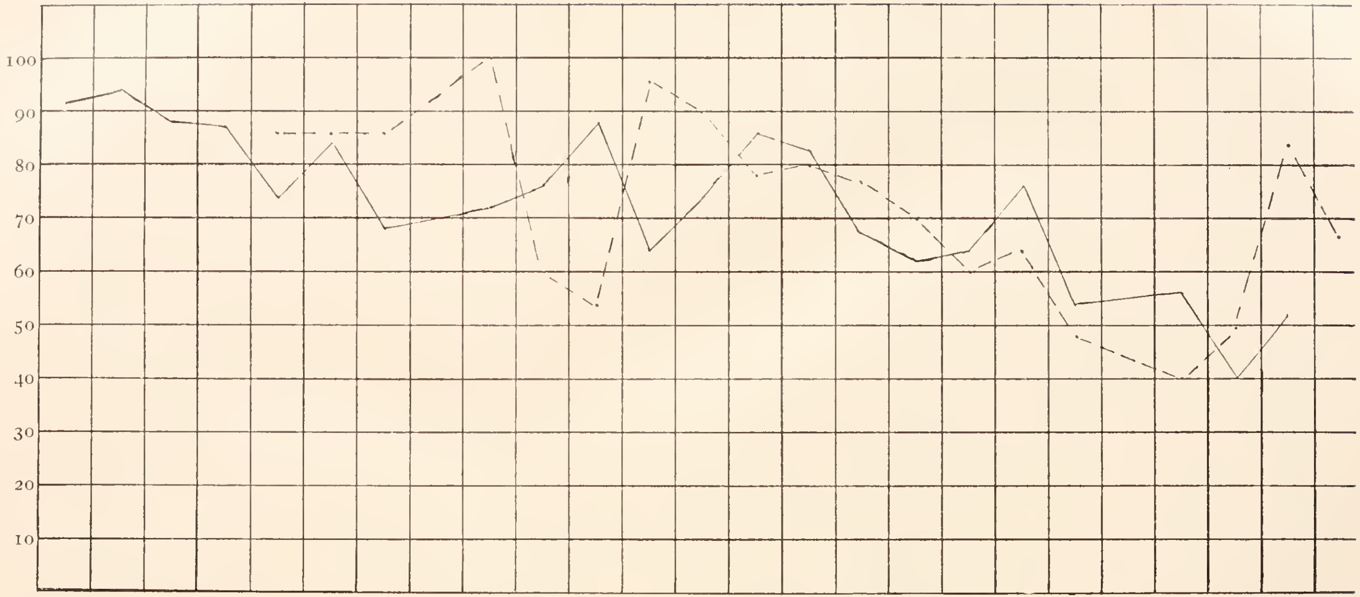

FIG. 1. Showing the result of rheotactic tests of stream isopods kept in the laboratory in stream water (solid line) and in pond water (broken line). As far as known the kind of water was the only factor varied. The abscissæ give the positive rheotactic reaction in percentage of the total trials for the day. Each space in the ordinates represents one day.

The rheotactic reaction of *Asellus* can be experimentally controlled by varying the oxygen and carbon dioxide tension within the limits found in the two classes of habitats. This is not true of similar variation in salt content, or in any other factor or group of factors yet studied.

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AN EXPERIMENTAL STUDY OF THE REACTIONS OF THE HORNED LIZARD, PHRYNOSOMA MODESTUM GIR., A REPTILE OF THE SEMI-DESERT.[1]

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PAGE.
I. Introduction 98
  1. General Habits 98
  2. Color and Color Changes 100
  3. Behavior in Captivity 101
II. Experimental Results 101
  1. Air Humidity Gradients 101
  2. Air Temperature Gradients 105
  3. Substratum Temperature Gradients 107
III. Conclusions 109
IV. Acknowledgments and Bibliography 110

I. INTRODUCTION.

The species of *Phrynosoma* which is the subject of these observations is one of the smaller and less conspicuous members of the genus, and is one of the commoner "horned toads" in New Mexico, which lies at the center of its rather restricted range (Ditmars, '08). This species, *Phrynosoma modestum* Gir. (Girard '53) has been confused to a certain extent in the literature (Herrick, *et al.*, '99) with another small member of the genus, *Phrynosoma platyrhinos* Gir. (Girard, '53) which extends into New Mexico from the northwest, but a reference to the original descriptions differentiates very clearly between the two groups.

1. *General Habits.*

Near Albuquerque, where the individuals observed were taken, the species is widely distributed over the sparsely vegetated "mesa" (Watson, '12) or clinoplane region varying in altitude from 1,500 to 2,200 meters and having a rainfall of approximately 30 cm. annually. Bailey ('13) gives the distribution of this animal as the Lower and Upper Sonoran Zones in New Mexico. In the valley of the Rio Grande, just below the "mesa" region, and in the Sandia Mountains bordering on the upper side the very different *Phrynosoma douglasii ornatissimum* prevails. This species is also found on the mesa but in much smaller numbers, and is said by Bailey (*l.c.*) to occur in the Upper Sonoran Zone. The whole region under consideration is given in his map as the Upper Sonoran Zone, with the exception of the Sandia Mountains, which include the Transition and Canadian Zones, in both of which the above variety of *P. douglasii* is found. Both mountains and valley are considerably damper than the mesa, the former on account of the greater annual rainfall, and the latter on account of the proximity of the Rio Grande and the high water table (10 cm. to 5 m. from the surface of the soil (Nelson, *et al.*, '14)). The distribution of these species seems to depend, not primarily on air temperature or on the factors which define the Life Zones of Merriam, although the upper limit of *P. modestum* approximately coincides with the upper border of the Upper Sonoran Zone, but upon factors which are in agreement in the river valley and the mountain range. Ants, the principal natural food of *P. modestum*, are more abundant in the mesa region, but this may be merely a secondary factor as far as distribution is concerned, as the animals eat and thrive well upon small beetles, plant bugs, grasshoppers, etc. The distribution of the species coincides with the grassy step formation of Watson ('12) falling in Shelford's ('13*a*) third division, *formations of savannas and grasslands.*

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[1] Contribution from the Zoölogical Laboratory of the University of Illinois, No. 86.

In captivity, it was observed that the lizards ate readily any living and moving insect or other animal of sufficiently small size. The animals have been observed even to ingest lead shot kept in motion by being rolled down an inclined plane, and occasionally snap at moving sand grains. The attempts of a medium-sized *P. douglasii* to swallow an earthworm of three times its own length were rather interesting. The lizard braced itself by means of its legs and attempted to pull the worm apart, but finally succeeded in swallowing it whole, shaking it in the process much after the manner of a dog shaking a snake. Large woolly caterpillars, grasshoppers of considerable size and other seemingly difficult objects were swallowed with comparative ease. The animals were never observed in the act of drinking and evidently obtained the amount of moisture required with the food.

Phrynosoma modestum is not, essentially, a desert and heat-loving species, although it seems more tolerant of desert conditions than *P. douglasii*. It appears in the greatest abundance in the early summer months, and during the early autumnal rainy season when the aërial temperature does not exceed 32° C., and when the temperature of the substratum in the sunlight is not over 38°. During these periods the animals actively move about all day, remaining during the night in protected nooks under bushes or in the burrows of other small animals, or occasionally buried in the loose soil. When the daily maximum temperature becomes greater the period of activity begins earlier in the morning and is terminated toward noon, when the temperature rises. During the heated part of the day the lizard is at rest, nearly, if not quite, covered by the loose soil or sand. The method by which this position is reached is very characteristic. The snout is directed downward and moved from side to side, the body extremely flattened, while the legs take part in a rapid horizontally clawing movement. The net result of this series of motions is to cover the animal with the loose soil, the depth varying according to the character of the soil, the individual, etc. When the temperature is high, sunlight is avoided and burrowing is more likely to take place in the shade of vegetation, a bank of soil or a rock. Other lizards have been observed to burrow in like manner as a means of escape from enemies.

2. *Color and Color Changes.*

The color of the animal generally resembles rather closely the color of the substratum, but this is not always the case. In general, individuals observed after a rain are darker in color (the soil is also darker) and very much lighter on a hot dry day. Experiments have shown, in the case of *P. douglasii*, that a rise in temperature, darkness or an increase in the evaporating power of the air causes a centripetal migration of the melanophoric pigment, while the opposite conditions induce a corresponding movement. Within the limits of the changes occurring in the habitat, the variation in the evaporating power of the air is the most potent of the above factors of color change. No direct connection between the color of the animal and that of the substratum has been confirmed experimentally.

3. *Behavior in Captivity.*

In captivity they are rather inactive, except when living food animals are introduced into the cage, when they may become very active, or when otherwise disturbed. They spend a great deal of time with the belly flattened to the ground, with the head lowered and the eyes closed. When the sun is not shining directly on the cage the majority of the animals are oriented toward the source of light (*e. g.*, a window) and often climb up on the side of the cage toward the light. On a cool morning they often orient themselves with respect to the sun so that the surface of the back is as nearly as possible perpendicular to the path of the rays. This is accomplished by tilting the body sidewise. When the air temperature is in the neighborhood of 30° or above direct sunlight is avoided,and the animals are active only when the cage is shaded. This is not in line with reported field observations on *Phrynosoma*, most of which seem to be based on the true desert species.

The horned lizards used for the following experiments were taken near the lower edge of the "mesa" (altitude 1,700 m.) just east of the city of Albuquerque, New Mexico, and near the campus of the University of New Mexico, about June 10, and shipped by express to the University of Illinois. They were kept, during the experiments, in two lots, one (cage No. 1) in a cage on the floor of a northeast second-floor room of the vivarium building, where the sunlight reached the cage but a few hours each forenoon. The evaporation in this cage, as measured with the Livingston porous cup atmometer averaged .5 c.c. per hour or 12 c.c. in 24 hours. The other lot was kept in a similar cage in the ground-floor greenhouse, where they were subjected to the direct rays of the sun until 2 P.M. The evaporation was here .8 c.c. per hour. The animals were fed black ants the greater part of the time, with occasional feedings of miscellaneous insects from net sweepings of vegetation near the building.

II. EXPERIMENTAL RESULTS.

1. *Air Humidity Gradients.* (*Evaporation Varied by Differences in Air Humidity.*)

A large number of experiments (120) were performed with the object of determining the optimum air evaporation and the reactions of the animals to air of different evaporating powers. The evaporation gradients were established by varying the humidity, the temperature and the rate of flow of the air to which the animals were subjected. The apparatus used in the gradient experiments was essentially the apparatus described by Shelford and Deere (1913) and Shelford ('14*a*) with the following modifications. The source of the air was the compressed air system of the University of Illinois. The measurement of the flow of the air was made more accurate by the insertion in the supply line of delicate draft gages described by Hamilton ('17). The gradient cages used in this series of experiments were also larger (45 × 8 × 5 cm.) to accommodate the larger animals, and the floors were covered with a layer of sand 1 cm. in thickness.

The experiments ordinarily covered each a period of twenty minutes, each space on the recording blank representing ten seconds, as indicated in the accompanying Chart I. The figures at the top of each column represent, respectively, the relative humidity, computed from readings of standard wet and dry bulb thermometers, and the evaporation per twenty-minute period, measured with porous cup atmometers, of the air supplied the corresponding third of the gradient cage. In the graphically recorded experiments an animal was placed in the cage, generally in the center section, and its movements recorded by means of tracings. In the statistically recorded experiments ten individuals were distributed through the cage and records of their positions made at the indicated intervals. Experiments designated by the same number were performed consecutively and with the *same* animal or group of animals. Otherwise the numbering is entirely arbitrary. For a more complete account of the method of recording, etc., see the explanation of Chart I.

In Experiments 12, 13 (Table I.) ten individuals were placed in the gradient cage and readings as to position were taken every thirty seconds, the final results being expressed in percentage figures. Experiment 12 was performed with individuals from Cage No. 1, in which the temperature varied between 24° and 35°, while those used in experiment 13 were from Cage No. 2, where the temperature rose to 40° during the warmer parts of the day. There was no marked difference in the behavior of the two

TABLE I.

SHOWING THE REACTIONS OF *Phrynosoma* IN GRADIENTS OF AIR HUMIDITY.

The method of procedure here was the same as that pursued in the experiments illustrated in Chart I., except that ten individuals were used in each experiment and numerical readings were taken at half-minute intervals. The summation of the results is indicated in numbers and in percentages at the foot of the columns. See explanation accompanying Chart I., and page 102 for discussion.

Minutes.	Experiment 12.			Experiment 13.			Experiment 15.		
	Humidity.			Humidity.			Humidity.		
	98%.	57%.	6%.	6%.	57%.	98%.	93%.	52%.	5%.
	0.3.	0.8.	2.0.	2.0.	0.8.	0.3.	0.2.	0.5.	1.8.
	3	3	4	3	3	4	3	4	3
1	4	2	4	2	5	3	1	6	3
	3	4	3	1	7	2	2	4	4
2	4	3	3	1	7	2	2	5	3
	4	4	2	2	7	1	3	5	2
3	5	4	1	4	5	1	3	5	2
	5	4	1	3	3	4	3	4	3
4	5	3	2	1	3	6	3	5	2
	5	4	1	2	4	4	2	5	3
5	4	6	0	2	2	6	2	6	2
	4	6	0	4	2	4	2	6	2
6	3	6	1	3	3	4	4	5	1
	5	4	1	1	4	5	4	5	1
7	5	4	1	2	3	5	3	5	2
	5	5	0	2	3	5	4	4	2
8	5	4	1	1	3	6	4	4	2
	5	4	1	3	2	5	1	5	4
9	4	5	1	3	5	2	3	5	2
	2	5	3	2	5	3	1	6	3
10	6	2	2	2	3	5	1	5	4
	4	3	3	4	4	2	0	5	5
11	4	4	2	4	4	2	1	5	4
	5	4	1	2	4	4	1	5	4
12	5	4	1	3	4	3	1	4	5
	3	5	2	5	2	3	0	5	5
13	3	5	2	3	3	4	3	3	4
	3	4	3	1	4	5	1	7	2
14	4	4	2	4	4	2	1	8	1
	3	5	2	4	3	3	2	8	0
15	4	4	2	3	2	5	1	8	1
	5	4	1	2	5	3	2	8	0
16	5	3	2	1	4	5	1	8	1
	6	3	1	3	2	5	2	6	2
17	5	2	3	2	4	4	1	8	1
	4	3	3	2	5	3	1	8	1
18	3	6	1	4	5	1	1	7	2
	5	4	1	1	7	2	0	9	1
19	5	2	3	0	7	3	3	7	0
	5	4	1	2	5	3	2	7	1
20	5	3	2	2	5	3	3	7	0
	172 43%	157 39%	71 18%	97 24%	160 40%	143 36%	89 22%	218 54%	93 24%

groups in this or in other experiments. In both cases the driest air was avoided, and the greatest number were found in the central portion of the cage, where the evaporation was .84 during a twenty-minute period. Many other similar experiments gave similar results.

Experiment 15.—This experiment did not show as much difference between the extremes but showed a great percentage in favor of the central section of the gradient cage in which the evaporation was much lower than in the preceding experiment (.5).

Summary of Air Humidity Gradient Experiments.

The animals ordinarily avoid air of excessive evaporating power, or, if they remain in such air, are stimulated in such a way that attempts to escape are made, or are stimulated to burrowing or digging activities. The net result of either reaction, if successful, is an escape from the unfavorable environment. As nearly as could be determined from the data at hand the optimum evaporation for the average animal lies between .5 c.c. and 1.0 c.c. expressed as the evaporimeter reading for a twenty-minute experimental period. There is a considerable variation in individual behavior and that of any individual will vary from time to time, due, presumably to changes in physiological condition. The normal evaporating power of the air in the natural habitat during the months of April and May varies from .15 c.c. twenty-four hour average on a rainy and cloudy day, to 1.0 c.c., on a bright clear day when the maximum temperature is in the neighborhood of 30°. Although day and night observations have not been made separately it is evident that the average evaporation for the daylight hours would be somewhat higher than the figures just given.

Animals kept for a long time in abnormally dry atmospheric conditions evidence a marked degree of uneasiness, indulge in many spasmodic movements, and finally either burrow or apply the body closely to the substratum. They are, however, able to endure long exposure to dry air without death. A 2.5 gram animal was exposed to a constant current of air with an evaporation of 2.4 c.c per twenty-minute period for a week before death took place. Death in this case was also probably largely due to starvation, as it was difficult to induce the animal to take food under the conditions of the experiment. The weight in this time was reduced to 2 grams.

The broad limits of the evaporation optimum make it rather unlikely that this factor is the principal one in determining the local distribution of the species. It would not be expected that this would be the case in a reptile from such a habitat, where the evaporating power of the air is subject to such great variations in the course of twenty-four hours. Reptiles, also, are, in general, especially adapted to withstand a considerable amount of drouth, the water loss through the integument being very slight and that through the feces and urine almost negligible, as compared to the similar losses of other animals.

2. *Air Temperature Gradients.* (*Evaporation Varied by Differences in Air Temperature.*)

Experiments with an air temperature gradient were carried out in the same apparatus as the previous experiments, the air passing through the cage being heated or cooled by passing through coils immersed in water of high or low temperature. The air came directly from the storage tank and was unmodified except as above noted and flowed at the same rate as in the humidity experiments. The experiments (34, *a*, *b*, *c*) summarized in Table II. together with the many others performed in the same manner show the optimum to be between 33° and 38° when the substratum is not cooled, *i. e.*, when the temperature of the substratum varies approximately with the air. When the substratum is cooled the optimum more closely approaches 30°, which corresponds, under the conditions of the experiment, to air with an evaporation of 1.0 c.c. for the experimental period. Both humidity and temperature gradients are essentially evaporation gradients and optima are most conveniently expressed in terms of evaporation (Shelford '13*b*).

TABLE II.

EXPERIMENT 34. SHOWING THE REACTIONS OF *Phrynosoma* IN AN AIR TEMPERATURE GRADIENT.

The temperatures of the thirds of the cage are in each case indicated at the top of the column. Readings were taken at one-minute intervals. For discussion see page 105.

Minutes.	Experiment 34*a*.			Experiment 34*b*.			Experiment 34*c*.[1]		
	Temperature.			Temperature.			Temperature.		
	29°.	33°.	38°.	38°.	33°.	29°.	37°.	29°.	27°.
1	3	3	4	3	4	3	3	2	5
2	4	2	4	3	5	2	3	2	5
3	4	2	4	3	6	1	3	2	5
4	4	2	4	4	4	2	3	4	3
5	3	2	5	4	4	2	4	4	2
6	3	2	5	4	5	1	4	3	3
7	3	4	3	4	5	1	4	3	3
8	3	4	3	4	5	1	4	3	3
9	3	4	3	4	5	1	5	3	2
10	3	4	3	4	5	1	5	3	2
11	3	4	3	4	5	1	5	3	2
12	4	3	3	4	5	1	4	5	1
13	2	4	4	4	5	1	4	5	1
14	2	4	4	4	4	2	4	6	0
15	2	4	4	3	5	2	4	5	1
16	2	2	4	3	5	2	4	5	1
17	2	4	4	3	5	2	4	5	0
18	2	3	5	4	5	1	5	5	0
19	2	3	5	4	5	1	6	4	0
20	2	3	5	5	4	1	6	4	1
21	2	3	5	5	4	1	6	4	0
22	1	5	4	5	4	1	6	4	0
23	1	5	4	4	5	1	7	3	0
24	1	5	4	4	5	1	7	3	0
25	1	5	4	3	6	1	7	3	0
26	1	4	5	3	6	1	7	3	0
27	1	4	5	3	6	1	6	3	1
28	1	4	5	3	6	1	6	3	1
29	1	4	5	3	6	1	6	4	0
30	1	4	5	6	3	1	6	4	0
31	1	4	5	6	3	1	6	4	0
32	2	2	6	5	4	1	6	4	0
33	1	2	7	5	4	1	6	4	0
34	1	4	5	5	4	1	6	4	0
35	1	4	5	6	3	1	5	5	0
36	1	4	5	5	4	1	5	4	1
37	1	4	5	5	4	1	5	4	1
38	1	4	5	5	4	1	5	4	1
39	1	4	5	6	3	1	4	5	1
40	1	4	5	5	4	1	4	5	1
	78 20%	144 36%	178 44%	167 42%	184 46%	49 12%	200 50%	153 38%	47 12%

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[1] Substratum cooled to 20°.

3. *Substratum Temperature Gradient.*

The gradient cage was placed in a water bath so arranged that hot water flowed into the latter at one end and cold water at the other, the water being directed backward and forward beneath the cage so as to produce a gradient in the temperature of the substratum. This experiment was conducted with still air in the cage, *i. e.*, with air of approximately the same temperature as the substratum and consequently varying in evaporating power, and with air of a uniform temperature and evaporating power flowing across the gradient. The results of the two types of experiment checked very closely, indicating that the temperature of the substratum is the dominant controlling factor under these conditions. Both show an optimum substratum temperature of 35° to 40°. In general the animals which had been kept at the higher temperature, *i. e.*, in cage No. 2, oriented themselves in the gradient more quickly than those kept at the lower temperature (Experiments 26, 27 *a*, *b*, Table III.). When the animals gathered in the region of the optimum temperature an increase or decrease of the temperature of the gradient caused a corresponding movement of all the animals, which were repeatedly driven from end to end of the cage in this manner. The lizards often burrowed at or near the upper limit of the optimum region. They also often burrowed when the temperature was reduced as low as 20°.

When the animals were placed in a cage the substratum of which was gradually heated, rapid movements, interspersed with digging reactions began at 35°, and as the temperature approached 40°, all animals attempted to burrow. However, under experimental conditions, it was impossible to heat the substratum without having the lower layers of the soil at least as hot as the top, and the animals did not complete the burrowing reaction, but again moved about the cage rapidly. Above 40°, evidences of extreme discomfort, such as wide-open mouth, spasmodic movements, etc., were evidenced.

When animals in a sand-bottom cage were subjected to a continuous blast of air of increasing temperature from above, so as to heat the surface of the soil without greatly increasing the temperature of the lower layers of the soil, they became very

TABLE III.

SHOWING THE REACTIONS OF *Phrynosoma* IN A GRADIENT OF TEMPERATURE OF THE SUBSTRATUM.

Temperatures are indicated at the tops of columns. Readings were taken at intervals of one minute. In Experiment 27 the figures in italics indicate burrowing on the part of the number of individuals so designated. For discussion see page 107.

Minutes.	Experiment 26.			Experiment 27.		
	Temperature.			Temperature.		
	19°.	39°.	43°.	25°.	40°.	52°.
1	3	4	3	2	6	2
2	1	5	4	1 *1*	8	0
3	1	4	5	1 *1*	8	0
4	1	3	6	*1*	8	1
5	0	4	6	*1*	8	1
6	0	4	6	*1*	8	1
7	1	4	5	*1*	8	1
8	1	5	4	*1*	9	0
9	2	5	3	*1*	9	0
10	3	4	3	*1*	9	0
11	4	3	3	*1*	*1* 8	0
12	4	3	3	*1*	*1* 8	0
13	2	5	3	Redistributed		
14	1	5	4	21°	36°	46°
15	1	5	4	4	2	4
16	1	5	4	4	3	3
17	1	5	4	3	4	3
18	1	5	4	3	4	3
19	1	5	4	3	5	2
20	1	6	3	3	5	2
21	2	5	3	3	6	1
22	0	7	3	1	9	0
23	1	5	4	0	9 *1*	0
24	1	5	4	0	9 *1*	0
25	0	5	5	0	9 *1*	0
26	0	5	5			
27	0	5	5			
28	1	4	5			
29	0	5	5			
30	0	5	5			
31	0	5	5			
32	0	4	6			
33	0	5	5			
34	0	3	7			
35	0	4	6			
36	0	3	7			
37	0	3	7			
38	0	4	6			
39	1	3	6			
40	0	4	6			

restless as the temperature of the soil approached 40°. Most of the animals burrowed, or at least flattened their bodies close to the substratum as the surface temperature reached two or three degrees above this. Unfortunately, in the apparatus available a higher air blast temperature than 43° could not be obtained.

The reaction to the temperature of the substratum, independent of the temperature and rate of movement of the air above and consequently independent of the evaporating power is the most definite and well defined response noted in the series, much more so than that in the air temperature and humidity experiments and must, therefore, represent an important factor in the daily and seasonal life of the animals. It is probable that this, and not the evaporating power of the air furnishes the most important direct stimulus determining the distribution and daily movements of the species. The surface of the soil in the Rio Grande valley region is cooler than that in the mesa habitat on account of its greater moisture content, and that in the mountains is cooler for this reason and also on account of the climatic variations due to higher altitude. The rise of temperature during the day stimulates the animal to burrow as a means of escaping the excessive heat. At least the surface temperature is of very great importance, as shown by the exceedingly consistent and regular behavior of the animals when this feature of their environment is varied. Unfortunately, but a single specimen of *P. douglasii* was available at the time the preceding experiments were conducted, and this one was taken at the same place as those of the more abundant species, *i. e.*, on the mesa. It would be of great profit to repeat this experiment with individuals of various species taken in entirely different habitats.

III. Conclusions.

1. The optimum evaporation rate of air for *Phrynosoma modestum* lies between 1.5 and 3 c.c. per hour, as measured by a standard atmometer. This is approximately the average evaporation rate of outdoor air in the natural habitat of the species, in sunlight, with a light breeze and a temperature of 30°. The reaction of the animals to a humidity gradient is not definite unless the gradient be a steep one. Daily variation in the habitat of the species is rather large.

2. The reaction to an air temperature gradient is more definite than the preceding. As temperature varies, the evaporating

power of the air also varies, and thus we get an evaporation gradient plus a temperature gradient, so that the gradient under these conditions is a double one. The optimum temperature is in the neighborhood of 30° when the humidity and rate of flow of the air is such as to produce an evaporation of 3 c.c. per hour, as measured by a standard atmometer.

3. Air humidity, air temperature and air current gradients are all essentially evaporation gradients, and the optimum in each case is a function of the evaporating power of the air.

4. The most definite and clear cut reaction in a gradient shown in this series of experiments is that in the substratum temperature gradient. This reaction is, to a great degree, at least, independent of accompanying differences in air temperature and humidity, or at least, the effect of these variations is overshadowed by the response to the gradient in substratum temperature. The optimum substratum temperature is rather definite—between 36° and 40°, and at the upper limit a very definite reaction (burrowing) takes place. This optimum is correlated with the other optima given above, in that the substratum temperature, under the conditions given in paragraph 1, is usually from five to ten degrees above the temperature of the air. Soil temperature (surface) is evidently of very great importance in determining details of seasonal and daily life, having as well its accompanying effect on the distribution of the species.

4. It is dangerous to ascribe to any one factor or group of factors the supreme rôle in determining the seasonal or general distribution of a species. The factors are certainly not the same for all species even in the same environment and before definite conclusions can be drawn a careful analysis of the habitat must be made, and experimental data must be obtained as to the reaction of the animals in gradients involving the factors capable of variation.

IV. Acknowledgments and Bibliography.

The writer is deeply indebted to Dr. V. E. Shelford, of the University of Illinois, for help and direction in the prosecution of the work and for assistance in the preparation of the manuscript. He is also indebted to Dr. M. M. Wells, of the University

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CHART I. Showing the reactions of *Phrynosoma* in gradients of the evaporating power of air. In the experiments illustrated this gradient was established by varying the humidity of the air with which the animals came into contact. The manner in which the gradient was established and the records made was as follows: A rectangular cage approximately 50 cm. × 10 cm. and 5 cm. deep was so arranged that currents of air from different sources could be directed across different sections of the cage. The openings were so arranged that the air passing across a given third of the cage was of uniform character throughout the experimental period, while that in each third differed as to humidity, but in no other way from that in the other sections of the cage. The animal could pass freely from one part of the cage to another. See text for more complete description of the apparatus. In the chart, each section between the numbered scales represents the record of a twenty-minute experiment, the distance between the scales representing the length of the cage, and the vertical length of the chart the time, twenty minutes, each division on the scale representing ten seconds. The curve in this space represents the movements of the animal under observation, and as the time-component is vertical and the space-component is horizontal, the parts of the curve more nearly horizontal represent the most rapid movements, while the vertical parts of the curve indicate that the animal was at rest during the length of time indicated on the scale. Thus, in chart 2*a* (the fourth from the left), the animal, introduced near the centre of the cage, remains in that position for approximately one minute, and then moves toward the left end of the cage, the movement occupying a period of ten seconds. The lizard remains in this position for a little over twenty seconds, and then moves toward the other end of the cage, where he arrives at the end of the second minute. Here he remains for three minutes, when he again moves toward the left end of the cage.

The figures accompanied by letters at the top of each section of the chart designate the number of the experiment. Experiments designated by the same number and consecutive letters were performed with the same animal and consecutively. Otherwise the experiment numbers are entirely arbitrary. The upper line of three figures in each section *e. g.*, 34, 19, 4, in 1*a*, indicates the relative humidity of the air passing through the corresponding thirds of the cage, as computed from standard wet and dry bulb thermometer readings. *S S S* in 1*c* and 5*c* indicates that in these control experiments the air was still and the same in all sections of the cage. The lower line of figures, *e. g.*, .5, .9, 1.5 in 1*a*, represents the standard atmometer reading, for a similar twenty-minute period, of the air passing through the corresponding third of the cage. Thus, in Experiment 1*a*, the relative humidity of the air passing through the left-hand third of the cage was 34 per cent., and the corresponding atmometer reading .5 c.c. In the middle third the relative humidity was 19 per cent. and the atmometer reading .9 c.c., while in the right-hand third the relative humidity was 4 per cent. and the atmometer reading correspondingly high, or 1.5 c.c. The rate at which the air passed across the cage was in each case 25 liters per sec.

Where the record is in the form of a dotted line, it indicates that the animal, in the corresponding period, either attempted to escape from the cage or to burrow in the sand on the cage bottom. In the records of experiments 3 and 4*a* the circles and the parallel lines following indicate that the animal burrowed under the sand and remained covered for the period indicated. In the record of experiment 5*a* the circle indicates that the animal was under the surface of the sand for a brief time only. For discussion of individual experiments see text.

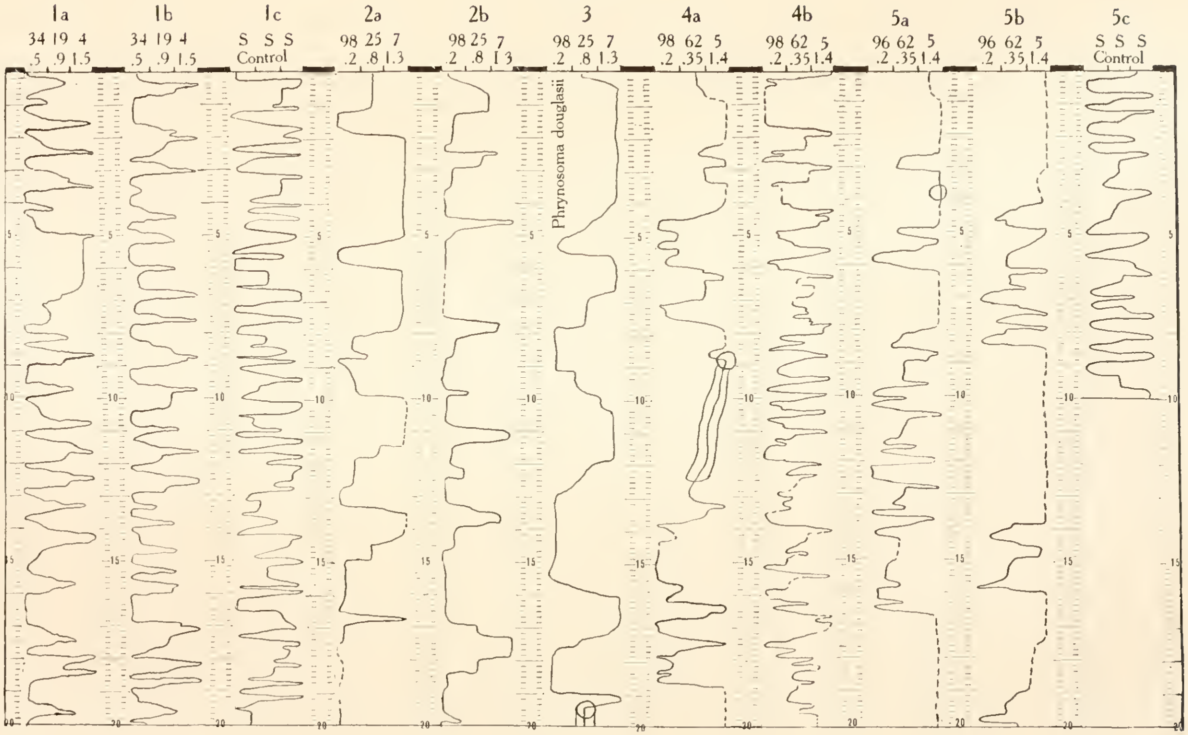
Experiments 1a, 1b, Control, 1c. (*Chart I*).—The tracing which is the graphic record of experiment 1*a* shows that the animal, for the first minute and a half, remained in the two portions of the cage where evaporation was lowest, then, at intervals about one minute made brief visits to the drier section. Following the fifth minute there is recorded a stay of over one minute in the driest third of the cage. Aside from this all the longer rest periods were spent in the opposite end of the cage. It will be noticed that, in general, the apices of the curves at the right are sharper than those at the left, indicating quicker turning in this region. In the first minute the animal turns away from the drier portion of the cage twice, once in the fifth minute, again in the eighth, tenth, sixteenth, eighteenth and twentieth minutes. There is only one turning back from the more moist regions, in the nineteenth minute.

The record of 1*b* is of almost the same character, with more avoidance of the dry end, as there are ten turnings during this twenty-minute period.

The control, 1*c*, in which the same animal was used shows somewhat greater activity than the two previous experiments, as the line crosses the area representing the cage a greater number of times. This curve, however, is not in the least one-sided, showing that the animal avoided no region of the cage. As indicated at the top of the chart, there was no air current through the cage in the control.

Experiments 2a, 2b.—In this experiment one end of the cage was supplied with air nearly "saturated" with moisture, as is indicated by the relative humidity of 98 per cent. and an atmometer reading of .2 c.c. During the first twenty-minute period, represented by tracing 2*a* the animal spent a little more time in the dry end than in the very moist region. During the second twenty-minute period (2*b*) the animal reached the dry third but six times, turning back almost immediately in

CHART I


1a
34 19 4
.5 .9 1.5
1b
34 19 4
.5 .9 1.5
1c
S S S
Control
2a
98 25 7
.2 .8 1.3
2b
98 25 7
.2 .8 1.3
3
98 25 7
.2 .8 1.3
4a
98 62 5
.2 .35 1.4
4b
98 62 5
.2 .35 1.4
5a
96 62 5
.2 .35 1.4
5b
96 62 5
.2 .35 1.4
5c
S S S
Control
Phrynosoma douglasii
5
10
15
20

each case. The longest stay in the dry end was in the eighteenth minute—about forty seconds.

Experiment 3.—This experiment is typical of those performed with *Phrynosoma douglasii*, and shows very little. The animal was inclined to be sluggish and finally, at the end of the period, burrowed in the middle section of the cage, where the humidity was 25 per cent. and the evaporation .8 c.c.

Experiments 4a, 4b.—This animal, beginning in the center section of the cage, moved, after nearly one minute, to the dry end, where attempts to escape, indicated by the dotted line, were recorded. It turned back from the medium section twice in the next two minutes, attempting to escape during the second stay in the dry section, and then spent nearly two minutes in the wet end, turning back twice from the medium air. A short visit to the dry end was then recorded, and then, at the beginning of the ninth minute, a marked escape reaction in the dry end, followed by a period of three and one half minutes, when the animal burrowed beneath the soil. While under the soil, the animal gradually moved toward the center of the cage and finally emerged there. Thereafter visits to the dry third were of very short duration until near the end of the period when escape at this end is again attempted. During the second period (*4b*) the animal was very active, and, except that, in general, the apices of the curves in the section of the diagram representing the dry end of the cage are more acute, indicating quicker turning here, very little can be said. There were many escape reactions in all parts of the cage.

Experiment 5a, 5b, Control 5c.—This experiment shows more definitely than any other the relation between stimulation to escape attempts and the length of stay in the dry air. It will be noted that escape attempts were recorded throughout each stay of any length in the dry air, and that, aside from these periods of stimulation the animal spent the greater part of the time in the center section of the cage. One short burrowing movement was recorded at the end of the fourth minute of the first period. The control, as elsewhere, showed a rather active movement from end to end of the cage, but no avoidance of any region.

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BIOLOGICAL BULLETIN

EFFECTS OF THE EXTIRPATION OF THE ANTERIOR LOBE OF THE HYPOPHYSIS OF RANA PIPIENS.

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These experiments were performed in the spring of 1916, although attempts had been made the previous year which failed because of faulty technique.

Adler ('14) removed the hypophysis ingrowth by using the electric cautery at a stage of 20 mm. length. This caused immense mortality and he was able to rear only 3 specimens. In these the hind limbs did not grow beyond the condition of mere buds and persisted in this condition long after metamorphosis of the controls and many months after the operation. He found that the growth of the thyroid gland was markedly retarded by the removal of the hypophysis and the amount of colloid was much reduced.

P. E. Smith performed quite similar experiments simultaneously with mine. Both of us gave simultaneous accounts of our respective researches before the San Diego meeting of the Western Society of Naturalists August 9 to 12. He published his results in *Science*, August 25, 1916, and in the *Anatomical Record*, October, 1916. I gave a preliminary account of my work in *Science* November 24, 1916. Since I have been delayed in making this more complete report, I can allow myself the privilege of drawing comparisons between Dr. Smith's work and my own.

My account of the experiments upon the hypophysis was delayed owing to the fact that I was absent from Lawrence during the summer and was unable to complete the study of my material until my return in September. I had also hoped to combine the account of these experiments with a full account of my work upon thyroid removal. Since six of the tadpoles in the latter experiment are still alive and the work upon the preserved material far from complete it seemed after all advisable to publish separate papers upon the two lines of work.

In the present series of experiments the removal of the hypophysis ingrowth was accomplished by making a frontal cut just beneath it and extending more than its entire length as shown in Fig. 1. It was then quite a simple matter to open up the wound and remove the hypophysis ingrowth by means of a spear-point needle. This was performed under a binocular microscope. The stage chosen for operating is that of 3.5 to 4 mm., the operation being performed more readily at this time than later because at this stage the ingrowth is compact and thick, but later becomes thinned out and more closely applied to the diencephalon, thus increasing the difficulty experienced in

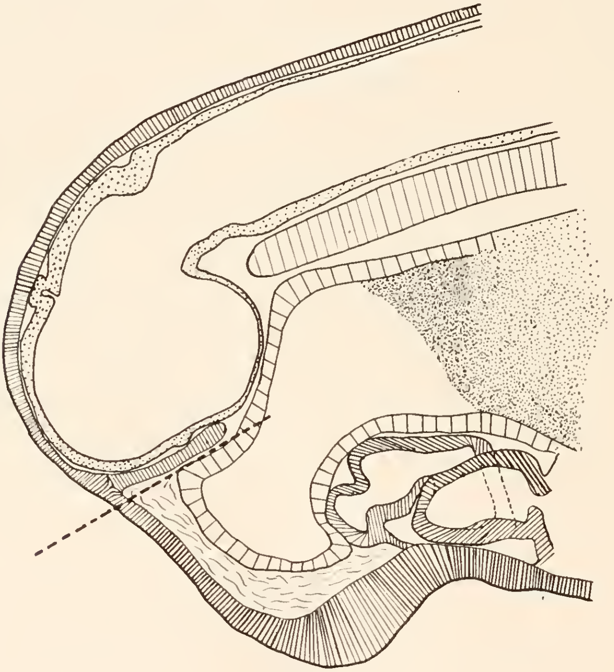

Fig. 1.

seeing and removing it. In each case it was broken away from the ectoderm to which it was still attached at the stage at which the operation was performed. In most instances the dorsal portion of the pharynx was partly removed; but, as will be seen later, this did not appear to affect the formation of the thymus glands.

The tadpoles recovered very quickly from the operation, healing of the wound taking place in the course of 20 or 30 minutes, after which the tadpoles became as active as ever and appeared to be quite normal. In about 1/3 of the cases the anlage of the upper part of the mouth was so injured by the operation that it developed imperfectly, hence these individuals were doomed to starve. Observations upon this point were made in the following lots as indicated:

Experiment.	Length of Tadpole at Time of Operation.	No. with Perfect Mouth.	No. with Defective Mouth.	Total No. Examined.
No. 23....	3.5–4 mm.	14	6	20
No. 24....	3 –3.5 mm.	22	11	33
No. 27....	3 –3.5 mm.	19	16	35

The operated tadpoles grew quite normally resembling the controls in every regard until 7 or 8 days after the operation when they were 8 mm. in length. They then underwent a striking color change from the earlier solid black to a bright creamy silver color, becoming really quite handsome creatures. In the controls many pigment cells are found in the lower layer of the epidermis, where they give off a rich network of processes. In sharp contrast to this is the fact that in the tadpoles deprived of the hypophysis only very seldom can a much contracted pigment cell be demonstrated in the epidermis. It seems quite certain that the pigment cells have migrated to deeper positions. In both the controls and the operated tadpoles they are found in great numbers on the surface of the skull, the inner surface of the gill cavity, the outer surface of the heart, within the brain, and spinal cord, upon the gills, in the pericardium, very extensively in the peritoneum, in the pronephros, liver, thyroid, lungs, intestine and walls of the blood vessels, but there is a constant difference in that they are expanded in the normal tadpoles and much contracted in the operated ones. It thus seems fair to conclude that in the absence of the hypophysis they migrate inward from the epidermis and that they contract throughout all parts of the body. In the more superficial cells of the epidermis scattered pigment granules are found here and there during later stages up to and beyond the 15 mm. stage. These persist in both the operated and control tadpoles. These observations and a careful study of the pigment cells convince me that there is no disappearance and bleaching of pigment granules as asserted by Smith. I differ from him further in my observations of the contraction of the pigment cells in the interior of the body in the operated tadpoles.

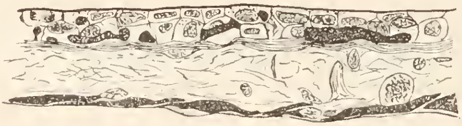

Fig. 2.

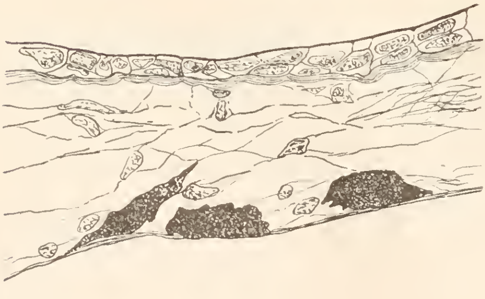

Fig. 3.

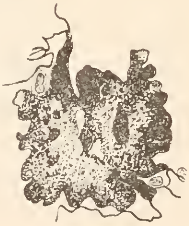

Fig. 4.

Figs. 2, 3, and 4 are all drawn to scale. Fig. 2 represents a lateral portion of the body wall of a normal control tadpole. The pigment cells can be clearly distinguished because of their darker color. Fig. 3 represents a similar portion of the body wall of a tadpole from which the hypophysis has been removed. This shows the absence of pigment cells in the epidermis and the contraction of those in the peritoneum. Fig. 4 shows a surface view of a pigment cell in the peritoneum of an operated tadpole.

We shall now take up a detailed account of the different experiments that bear upon the subject of this paper.

No. 13.—April 9 and 10, 1916. Length at time of operation 5.5 mm. These tadpoles were in too advanced a stage of development to give satisfactory results. It was impossible to clearly see the hypophysis ingrowth because it had become so much broadened and flattened. The ventral surface of the fore-brain was scraped and frequently injured in the process. Under this heading are included three series of experiments.

(*a*) April 9. 14 operated, rather crudely. 10 remained alive at the end of 9 days. These were discarded because of later improvements in technique.

(*b*) April 9. 12 operated. The work was much more carefully performed than in (*a*). The under side of the fore brain was carefully scraped and all specimens that showed injury to the brain were discarded. 6 remained alive at the end of 11 days when they were preserved for sectioning.

(*c*) April 10. 20 operated. In this case the floor of the diencephalon behind the optic stalks was removed in order to insure the complete removal of the hypophysis which in itself is quite hard to recognize at this late stage. Only 3 remained alive at the end of 6 days and they were distorted.

These experiments were made at entirely too late a stage of development to be successful. They are merely given to show the stages through which the work progressed.

No. 14.—(*a*) April 10. 12 operated. 3 alive at the end of 6 days. Length at time of operation 4 mm. This was the first really successful experiment. This unfavorable result was purely due to neglect owing to the press of work in later experiments. The remaining specimens were killed.

(*b*) April 11. 65 operated. Tadpoles kept in a finger bowl for 2 days when it was found that 20 showed abnormalities due to lack of oxygen. These were isolated, some dying within a few days while others partly recovered. These were later discarded. Of the remaining 36 healthy tadpoles 31 were alive at the end of 26 days. During this time they were kept in soft cistern water. They were fed algae which helped to oxygenate the water in the aquaria. The close quarters of the aquarium bowl in which they were kept no doubt retarded their growth. They showed the following characters as to color and size:

	No. Specimens.
Dark but with a more silvery cast than usual — 11, 12, 12, 13 and 14 mm. — one each	5
Silvery runts under 11.5 mm.	16
Silvery specimens of larger size—11.5–13.5 mm.	7
14, 16, and 18 mm.— one each	3
All but two of the small ones had a poorly formed mouth. Killed May 7	31

(*c*) April 12. 51 operated. Of these, 8 were used for experiment 17. Of the remaining 43, two died on April 29; one on the 30th and then the mortality became very marked so much so that 31 died in the succeeding 5 days. On May 5 the remaining 9 were preserved. They had lived 23 days. These were kept in city water in roomy kitchen sink aquaria from April 18 on. The high mortality in comparison with that of lot 14 (*b*) is really very striking.

No. 17.—April 12 hypophysis removed, April 13 thyroid also removed. Kept throughout in a glass bowl aquarium in soft cistern water. At the end of 31 days two were dead, the remainder were killed. Of these killed May 13, 5 were light and 1 dark.

No. 19.—April 14. Length at time of operation 5 and 6 mm. 21 operated. The operation was performed upon specimens too far advanced. They showed a high rate of mortality and were soon discarded.

No. 22.—April 20. Stage at operation varied 3.5–4.5. 40 operated. Two died soon after. Divided into two lots of 19 each. Lot (*a*) was kept as before, in lot (*b*) the thyroid gland was also removed.

In Lot (*a*) 3 dead by May 1.

In Lot (*b*) 3 dead by May 3.

All dead in few succeeding days. Data not complete. On April 28 both (*a*) and (*b*) equally showed the characteristic color change due to removal of the hypophysis proving that this is not due to any lack of balance between these two glands.

Controls kept in same sink showed no mortality.

No. 23.—April 22. Stage of operation 3.5–4 mm. 42 operated. Great care was exercised to avoid injury to the brain.

April 29. 7 died.

April 30. 5 died.

May 1. 5 died.

May 2. 5 died.

On May 2, 19 were nearly dead—only the faintest heart beat could be seen and only 1 was still active. The 20 were killed on that date.

Controls kept in neighboring sink showed no mortality.

No. 24.—April 23. Stage of operation 3–3.5 mm. 51 operated. Great care was exercised to avoid injury to the brain.

April 29. 1 died.

May 1. 3 died.

May 2 and 3. 36 died.

Only 11 remained alive at this time. Controls kept in neighboring sink showed no mortality.

No. 27.—April 30. Length at time of operation 3.5 to 4 mm. 103 operated. 3 died within 24 hours. Care was exercised to avoid injury to the brain. Divided into two lots.

(*a*) 30 kept in aquarium bowl in soft water for 10 days; there was no mortality during this time. Transferred to sink with city water on May 10. May 21 all dead but one that was dark in color.

(*b*) 70 kept in sink with city water from outset.

May 4	3 dead
" 6	1 "
" 16	10 "
" 17	10 "
" 18	13 "
" 19	7 "
" 21	8 "
" 24	9 "
June 1–10	3 "
	64 dead
Missing	2
Killed June 1	1
Killed June 10, of these, 1 black, 1 gray, 1 white (small)	3

Controls showed no mortality during this time.

No. 28.—May 8. Length at time of operation 3.5–4 mm. 30 operated. On June 10 4 remained alive—two killed and two preserved. One of these lived until July 2 while the other lived until Aug. 30th. The former was killed when 24 mm. long. The latter reached a length of 30 mm. It was extremely active and of a bright silver color. It was unfortunately lost.

Table Showing Mortality in Various Experiments.

In Soft Water.

No. of Exp.	Time of Starting.	Termination of Experiment.	No. Days.	No. Tadpoles in Exp.	No. Dead.
14 *b*	April 11	May 7	26	36	5
17	April 12 and 13	May 13	30	8	2

In City Water (Hard).

No. of Exp.	Time of Starting.	Termination of Experiment.	No. Days.	No. Tadpoles in Exp.	No. Dead.
14 *c*	April 12	May 5	23	43	34
23	April 22	May 3	11	42	41
24	April 23	May 3	10	51	40
27	April 30	June 1	31	103	97
28	May 8	June 10	32	30	26

We can see in these experiments the serious effect of the Lawrence city water upon tadpoles deprived of the anterior lobe of the hypophysis. An analysis of the water made June 1 shows the following mineral content:

In terms of mg. per liter.

Total solids	584.0	
SiO_2	26.2	
Fe_2O_3—A(2O_2	2.6	fluctuates greatly from this figure to 13 mg.
Ca	114.8	
Mg	16.0	
Na plus K	62.8	
Cl	78.0	at no time during experiment in free condition.
SO_4	58.8	
HCO_3	388.0	

The Fe is the only ingredient that shows frequent fluctuation. It is interesting to note in this connection the results of a test control experiment.

No. 27 X.—On May 1 tadpoles from the same batch used in 27 were employed. These had been held in their development at the operative stage by being kept upon ice. Soon after the operation they were placed in city water.

(*a*) 16 tadpoles were cut along a plane lying just above the hypophysis ingrowth. In the majority of cases the brain was touched. 4 died on May 5.

(*b*) 15 tadpoles were cut in the usual manner in preparation for removal of the hypophysis ingrowth but as in the preceding (*a*) the hypophysis ingrowth itself was left intact.

After (*a*) and (*b*) had been kept separate for 10 days with the loss of 4 in (*a*) they were then combined.

Following this, deaths occurred as follows:

May 18	1 dead
“ 21	1 “
“ 24	2 “
“ 25	5 “
“ 25–June 9	6 “

Thus on June 9 14 were still living and healthy. They were then killed. It is interesting to compare the results obtained here with those found in the above table. It is seen that by deducting the 4 that died in the first 5 days, as a result of serious injury there were 28 that had a good start without any mortality until May 18; 14 or $\frac{1}{2}$ of these survived 40 days later. Unfortunately a study of the mouths of these tadpoles was not made. If it had been done it might have been possible to explain some of this mortality as a result of starvation due to imperfect mouth development resulting from injury. A series of measurements made on May 16 showed 12 to be below 11.5 mm. in length while the maximum length was 13.6 mm. The very smallest, 8.7 mm., was of a silvery color due supposedly to the accidental removal or injury of the hypophysis ingrowth.

This leads to the important point that this was the only case of a color change in the 28 specimens, thus showing that the absence of the hypophysis and not the mere wound itself is the cause of the color change. Along this line may be mentioned that in nearly every experiment where removal of the hypophysis was practiced one or two operated tadpoles failed to show the color change characteristic of the others. Several of these were sectioned and in each case it was found that there had been failure to remove the hypophysis. There is thus no doubt whatever that the absence of this gland is the cause of it. The nature of this color change will be discussed later. The operation and the absence of the hypophysis have their influence upon the size of the tadpoles.

Lots 27(*a*) and (*b*) also 27 X (control operated) (*a*) and (*b*) were measured on May 16 giving the following results:

Lot.	Maximum.	Minimum.	Average.	No. Specimens.
(27 *a* (deprived of hypophysis)	12.0 (dark) 10.6	8.3	9.43	15
27 *b* (deprived of hypophysis)	15.3	8.9	10.74	45
27 X *a* and *b*—operated control	13.6	8.7 silver one 9.6 dark	11.53	29
Control (none operated)	20.4	11.6	16.95	30

It is to be remembered that 27 X *a* and *b* was performed 1 day later than 27 *a* and *b*.

It is thus seen that although the operated controls did not grow as rapidly as the unoperated controls they grew decidedly more rapidly than did the operated tadpoles. It is also seen that a few of the largest operated tadpoles showed greater size than the smaller controls. It is difficult to explain this. It may be partly due to a difference in vitality.

The serious mortality and delay of growth are in sharp contrast with the results of the equally severe operation of removal of the thyroid anlage. This was performed by making a transverse cut between the heart and the thyroid, and then picking out the latter by means of a needle. The cut was quite as large as that made for the removal of the hypophysis and produced a considerable loss of blood, yet there was no greater mortality in the operated tadpoles than among the controls. In time they appeared precisely like the normal controls and remained so until the legs began to grow in the controls while remaining at an early stage of development in the thyroidless tadpoles.

Returning to the experiments upon removal of the hypophysis, upon the question of mortality my results are quite at variance with those of Dr. Smith who found that there was even less mortality among his operated tadpoles than among his controls. I feel that this difference between his results and mine may well be explained by the fact that our material was reared in a different water supply. It is quite significant that lots 15 *b* and 17 raised in aquarium jars with soft cistern water lived with very little mortality until I killed them. They had ceased to grow because of overcrowding in narrow quarters. The serious effects of our water supply upon normal tadpoles were noted in a series of experiments upon removal of the thyroid, to be published later. In these both the thyroidless tadpoles and their controls began to show a remarkable twist in their tails when they reached a length of about 25 mm. on June 8, 57 days after the operation. At this time they began to show a high mortality. This was even more marked in the controls than in the operated ones. Only a very few that had been deprived of the hypophysis remained alive at this time and they were not far enough advanced to show these modifications.

This whole matter will be the subject of extensive work next spring in an effort to determine whether the high mortality among the tadpoles deprived of the hypophysis is due to a resulting susceptibility to some specific substance in the water. We should be able by these means to trace out some features in the influence of the hypophysis upon metabolism.

There was marked retardation in the development of the limb buds. This was quite evident in the 24 mm. specimen killed July 2. The operated tadpole of this stage had limb buds 234 micra while in the control they were 532 micra in length. According to the reports of a friend who kept the 30 mm. tadpole under observation up to the time of its death, August 30, the limb buds remained strictly rudimentary. At this time the controls had long before undergone metamorphosis. In these regards my work is in complete accord with that of Adler and Smith, namely, the removal of the hypophysis prevents the hind legs from developing beyond a rudimentary condition, and causes the tadpoles to remain in a larval state.

The internal structure of the few operated tadpoles that reached sufficient size was studied in comparison with the controls. The stages thus investigated were of 15.5 mm., 16.5 mm., 21.5 mm., and 24 mm. length. One that reached 30 mm. length unfortunately died without being preserved. Especial attention was given to a study of the thyroid and thymus glands and to the gonads. A more cursory examination of other organs was made, but failed to show any noteworthy features.

In measuring the different organs there were in each case three measurements made in portions that show approximately maximum thickness. Such measurements were made in two dimensions in each case. The averages computed from them are as follows:

16.5 Mm. Stage.

	Thymus.			Thyroid.			Gonad.		
	Leng., μ.	Bread., μ.	Thick., μ.	Leng., μ.	Bread., μ.	Thick., μ.	Leng., μ.	Bread., μ.	Thick., μ.
(Control, indif. sex)									
Left	420	248	362	150	114	21	710	78	78
Right	560	234	476	150	78	21	610	64	85
(Operated, indif. sex)									
Left	430	341	532	150	106	44	790	64	135
Right	420	341	504	120	114	35	630	94	99
21.5 Mm. Stage.									
(Control, female)									
Left	660	327	461	220	142	21	1,150	85	170
Right	670	335	639	210	111	22	990	135	142
(Operated, male probably)									
Left	460	369	639	190	170	43	930	59	67
Right	470	412	882	200	170	43	790	67	75
24 Mm. Stage.									
(Control, male probably)									
Left	840	426	639	290	177	57	1,170	114	99
Right	880	426	639	300	149	57	1,020	114	114
(Operated, male)									
Left	790	525	852	250	270	28	660	114	170
Right	860	504	696	210	362	28	840	114	92

Table Showing the Number of Follicles in the Thyroid Gland and the Average Diameter of the Colloid Secretion Masses.

16.5 Mm. Stage.

		Number of Colloid Masses.	Diameter of Colloid Masses.
Control	Left	20	
	Right	12	
Operated	Left	15	
	Right	16	
21.5 Mm. Stage.			
Control	Left	16	22μ
	Right	13	
Operated	Left	7	11μ
	Right	8	
24 Mm. Stage			
Control	Left	21	29μ
	Right	25	
Operated	Left	20	18μ
	Right	17	

It is clearly evident from the above figures that tadpoles deprived of the hypophysis produce far less colloid substance in their thyroid glands than do the normal controls. This is a markedly constant point of difference. This would seem to be an index of its secretory activity in the two cases; although the recent work of Bensley would cast doubt upon the value of the colloid accumulation as an index of the secretory activity of the thyroid.

In the 16.5 mm. and 21.5 mm. stages, the thyroid is actually larger in the operated individuals than in the corresponding controls while in the 24 mm. stage the thickness—dorso-ventral dimension—is on the contrary almost twice as great in the control as in the operated specimen. The material is too scanty to enable one to draw positive conclusions upon this score from my work alone. It may be well to point out however that Adler, Smith and myself are in agreement regarding the effect of the removal of the hypophysis upon the development of the thyroid gland and regarding the relatively small amount of colloid produced in them.

I should hesitate to attribute the failure of the limbs to develop in operated tadpoles to the lessened activity of the thyroid resulting from the absence of the hypophysis. This question must be tested by more crucial experiments than any as yet applied.

In general the thymus gland is larger in the operated tadpoles than in the controls. The differences are not constant nor are they striking in the face of the general variability of this organ. It only seems safe to state that the thymus is not adversely affected in size or structure by the removal of the hypophysis.

The gonads show a large amount of variation in size and form. The absence of the hypophysis does not produce any apparent modification in them in the stages studied. The germ cells, sex cords and the various structures of the gonads appear to be quite unmodified by the operation. The operated tadpoles of 21.5 mm. and of 24 mm. stages after sexual differentiation are both males. It is thus impossible to tell whether there is any difference in the effects upon the two sexes. This point of the effect upon the gonads must be carried out in future experiments reaching to much later stages and involving far larger numbers of tadpoles.

SUMMARY.

1. The removal of the anlage of the anterior lobe of the hypophysis early causes the pigment cells to contract, and those of the epidermis to withdraw from it into the interior. This takes place while the gland shows little or no apparent histological differentiation.

2. Evidence has been given to show that the absence of the hypophysis in tadpoles makes them highly susceptible to unfavorable chemical conditions of the water. This will be tested out specifically in later experiments.

3. The absence of the hypophysis causes—either directly or indirectly—a failure to undergo metamorphosis especially evident in the absence of limb growth beyond a very rudimentary condition.

4. The absence of the hypophysis causes a marked diminution of colloid formation in the thyroid gland and in the stage of 24 mm. involves a retardation in its growth.

5. During the stages studied the absence of the hypophysis produced no noticeable effects upon the thymus gland nor upon the gonads.

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TEMPERATURE-COEFFICIENTS IN THE ACTIVATION OF STARFISH EGGS BY BUTYRIC ACID.

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INTRODUCTORY.

In experiments performed at Woods Hole during the summers of 1915 and 1916 it was found that unfertilized starfish eggs may be completely activated—so that with normal eggs 95 per cent. or more form blastulæ—by a single exposure to a weak solution of butyric acid (in sea-water or Van't Hoff's solution) at room temperature.[1] To secure this result with a given solution of acid all that is required is that the duration of the exposure should be definite within somewhat narrow limits. Exposures briefer than this optimum cause incomplete activation, which may fail to carry the egg beyond membrane-formation and a few early cleavages; in such cases the activation may be completed and the egg rendered capable of advanced development by a second properly timed exposure to the same solution. Over-exposure directly injuries the eggs and impairs or destroys their power of development. Corresponding to each concentration of acid within a wide range (.0005 *n* to .006 *n*) there was found a well-defined optimum duration of exposure; this duration was approximately inversely proportional to the concentration of acid.

These facts, especially the direct proportionality between the concentration of acid and the speed of the activation-process, indicate that a chemical interaction between the acid and some unknown egg-constituent (probably surface-component), rather than a purely physical effect, is the critical or determinative event in this type of activation. Apparently during the period of exposure this interaction continues uniformly, at a rate determined by the temperature, concentration of acid, and the structural conditions in the egg-system, until a certain definite quantity of reaction-product is formed; this is the critical quantity required to enable the egg to continue its development to an advanced stage; and if the reaction is then arrested by returning the eggs to sea-water, activation is found to be complete and development continues normally. If, however, the exposure has been too brief, so that the quantity of reaction-product formed is insufficient, activation is only partial and development ceases at an early stage. Similarly, over-exposure leads to an excessive accumulation of the reaction-product—a condition also unfavorable to development. According to this hypothesis, the activation-process consists essentially in the production of a definite reaction-product, which may be called the activating substance. Since the rate at which the butyric acid takes part in the process is directly proportional to its concentration, it is evident that the interaction has the character of a monomolecular reaction: *i. e.*, the product of the concentration into the time required to produce a given quantity of reaction-product is constant (qt = const.). The quantity of egg-constituent transformed into activating substance in unit time is a direct linear function of the concentration of butyric acid.[1]

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[1] BIOLOGICAL BULLETIN, 1915, Vol. 28, p. 260; *Journ. Biol. Chem.*, 1916, Vol. 24, p. 233.

Some further test of this hypothesis has seemed desirable, since the objection is possible that the activating effect depends simply upon the entrance of a certain critical quantity of acid into the egg. According to the general law of diffusion, the rate of such entrance would be proportional to the difference in the concentration of acid between medium and cell-interior; hence the time required for the entrance of the activating quantity of acid ought to vary inversely with its concentration in the medium. No inference as to the mode of action of the acid would then be possible from the above facts alone. A possible means of deciding between these alternatives lies in determining the manner in which the rate of activation in a given solution of acid is influenced by change of temperature. The temperature-coefficient of reaction-velocities at ordinary temperatures is usually much greater than that of diffusion;[1] hence if the simple rate of diffusion into the egg is the essential factor determining the rate of activation, the effective times of exposure should be only slightly altered by changes of temperature. On the other hand, if the rate of entrance is itself unimportant,[2] and the essential action of the acid consists in forming a chemical compound, the rate of activation should be influenced by temperature in the manner characteristic of chemical relations, *i. e.*, doubled or tripled by a rise of 10°.[3]

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[1] The destruction of an inhibiting substance by combination with the acid might be supposed to underlie the activation effect, but in this case the rate of destruction would not be uniform, but would fall off with the progressive disappearance of the inhibiting substance. All of the evidence indicates that the rate of activation is uniform and a direct function of the concentration of acid.

EXPERIMENTAL.

In the experiments described below the rates of activation have been determined for a considerable range of temperatures, 2° to 28° (with intervals of 2°), using a single concentration of butyric acid throughout, viz., .006 *n* in sea-water (6 c.c. *n*/10 acid *plus* 94 c.c. sea-water). In each series of experiments eggs from the same lot—usually taken from a single animal to insure uniformity of condition—were exposed simultaneously to this solution at the two (or three) temperatures under comparison. Usually in any single series two temperatures differing by 2° (*e. g.*, 10° and 12°) were chosen; sometimes three temperatures were used, in case this could be done without interfering with manipulation. The procedure was simple: the eggs, after washing thoroughly in sea-water, were placed in two (or three) small beakers, each containing a thermometer; after the eggs had settled the sea-water was removed as far as possible; then the solutions of butyric acid, at the temperatures chosen for the experiment, were added, first to one beaker, and then, after a short definite interval (*e. g.*, 10 seconds), to the second. The temperature of each beaker was kept constant during the period of the experiment by immersion in a water-bath of the same temperature; and at definite intervals eggs were transferred to finger-bowls containing sea-water. A series of dishes, each containing eggs exposed to .006 *n* butyric acid for a definite length of time (*e. g.*, 1 m., 2 m., 3 m., etc.), was thus obtained for each temperature. The intervals between successive transfers varied from ½ min. at the higher temperatures (22° and over) to 3 or 4 minutes at 8° to 12°. The condition of the eggs in each dish and the proportion developing to larval stages were determined later. The differences between successive members of such a series are usually distinct, and the proportion of favorably developing eggs is always found to increase progressively up to an optimum, after which it declines. The determination of the optimum duration of exposure thus presents no difficulties. As a rule one dish shows distinctly more numerous larvæ than those on either side; but with favorable lots of eggs and brief intervals between the transfers it is often found that at the region of optimum two or even three successive exposures give equally good results.

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[1] A list of temperature-coefficients of different physico-chemical properties and processes is given in Snyder's paper, *Amer. Journ. Physiol.*, 1908, Vol. 22, p. 309. The values given for diffusion-processes are probably too high; Öholm, working in Arrhenius' laboratory, finds for eight substances, including salts, alkalis and acids (HCl and CH_3COOH) Q_{10} values ranging from 1.19 to 1.28. *Cf.* Öholm, *Zeitschr. f. physik. Chem.*, 1905, Vol. 50, p. 309 (cited in Lewis' "System of Physical Chemistry," Vol. 1, 1916, p. 428).

[2] As would be the case if the rate of entrance were decidedly more rapid than the rate of participation in the activation-process.

[3] This is the usual coefficient for the range of temperatures under consideration in physiological processes. In general the Q_{10} values for chemical reaction-velocities decrease as the temperature rises; for a study of this phenomenon *cf.* Cohen Stuart, *Proceedings of the Royal Society of Amsterdam*, May 23, 1912, p. 1159. Snyder notes that in physiological processes a decrease of Q_{10} at higher temperatures is also frequently found (*Amer. Journ. Physiol.*, 1911, Vol. 28, p. 167). In the activation of starfish eggs by butyric acid, however, the reverse is the case, as will be seen below.

At temperatures below 8° it is necessary to distinguish between the effects due to the butyric acid, and those produced directly by cold alone. Prolonged exposure to temperatures of 6° and lower has a well-marked activating influence on star-fish eggs.[1] At these temperatures butyric acid solutions were found to act *more* rapidly than at temperatures several degrees higher (8° to 12°); this increase in the rate of activation with fall of temperature indicates the entrance of *cold* as a factor; we have then to deal with a summation of two activating influences, cold and acid. Between 8° and 28° the influence of temperature alone is insufficient for activation. Above this range temperature conditions again become effective; simple exposure to sea-water at 30° and higher for the proper length of time complete activation.[2] There

[1] *Cf.* Greeley, *Amer. Journ. Physiol.*, 1902, Vol. 6, p. 296.

[2] R. S. Lillie, Biological Bulletin, *loc. cit.*

are thus two ranges of temperature, one below 8°, the other above 29°, exposure to which may induce activation. Eggs may be exposed to sea-water at 28° for 45 minutes without showing any external signs of activation, such as membrane formation, although some latent effect is probably produced.[1] At about 29° definite activation effects begin to appear, and at 30° these are well marked.[2] It is found, however, that at temperatures considerably below the range of heat-activation the action of butyric acid is accelerated to a disproportionate degree by slight rise of temperature; the temperature-coefficient of this action, at first of the order 2–3.5, begins to show decided increase at 20° or even lower, and a rise from 26° to 28° approximately doubles the rate of activation (see Table II). This behavior indicates that at higher temperatures the combination of the acid is facilitated by some secondary change in the egg-system due directly to the temperature. Apparently the structural conditions under which the activating reaction proceeds are modified in a definite manner by a rise in temperature. The indications are that at 30° and higher the structure is so altered that acids formed in the egg itself (*e. g.*, lactic)[3] become free to combine and cause activation· this hypothesis explains why heat-activation and acid-activation exhibit so many features in common, in particular closely similar relations between duration of exposure and degree of activation.[4] The high temperature-coefficients of acid-activation above 20° are thus probably the expression of a superposition of two effects, one a structural alteration due directly to high temperature, the other a chemical combination of the acid with some cell-constituent. The second is the critical event in activation, but its rate is determined not only by the temperature but by the structural conditions in the egg-system.

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[1] This is indicated by the marked acceleration in the rate of acid-activation at or near this temperature. See below, p. 142.

[2] *Loc. cit.*, p. 268.

[3] This acid is formed rapidly in many cells at higher temperatures, as seen in the phenomena of heat-rigor.

[4] *Cf.* my recent paper, BIOLOGICAL BULLETIN, *loc. cit.*, p. 282: also *Journ. Biol. Chem.*, *loc. cit.*, p. 234.

Another characteristic effect which is produced in starfish eggs both by temporary warming and by temporary exposure to butyric acid solutions is the prevention of the maturation-process. Eggs exposed for some minutes to warm sea-water (*e.g.*, 32°), within a few minutes after removal from the animals—*i.e.*, before the dissolution of the germinal vesicle has begun—remain permanently immature (*Journ. Exper. Zoöl.*, 1908, Vol. 5, *cf.* p. 400). The same is true of eggs exposed similarly to butyric acid solution. This identity in the physiological effect produced by the two apparently quite different treatments is a further indication that these high temperatures act by causing the production of acid within the egg.

The action of butyric acid at 6° and lower also represents a summation of two activating influences, cold and acid. I shall deal with this case separately, and shall consider first the temperature-coefficients of the action of butyric acid between 8° and 28°, temperatures which by themselves have no activating effect.

(*a*) *Experiments with Butyric Acid Solution at 8° to 28°.*

It was shown in my two preceding papers[1] that the degree of activation (as indicated by the proportion of favorably developing eggs) resulting from exposure to a given solution of butyric acid at ordinary temperatures increases with increasing time of exposure up to an optimum. Exposures longer than this optimum are injurious and beyond a certain maximum simply cause cytolysis without activation. This rule holds for the action of butyric acid at all temperatures. The interval between the minimum for visible activation (membrane-formation alone) and the optimum decreases rapidly as temperature rises; at 8° this interval is approximately forty minutes, at 18° six to eight minutes, and at 28° less than one minute. A curve relating degree of activation (the percentage of eggs forming blastulæ) to time of exposure may thus be constructed for each temperature. The form of this curve appears to be the same at all temperatures, although as just indicated the time-range which it occupies is shorter at higher temperatures. This behavior is consistent with the foregoing hypothesis that activation depends upon the progressive formation of a reaction-product (acid *plus* egg-substance = activating-substance) to a critical local concentration or quantity, the rate of formation being a function of temperature and concentration of acid.

According to this hypothesis, the interval between the beginning of exposure and the optimum ought to vary with temperature in accordance with the usual temperature-coefficient of reaction-velocities ($Q_{10} = 2 - 3.5$). The experiments described below show that this is approximately the case for temperatures between 8° and 18°;[1] but with further rise in temperature the Q_{10} values increase at a disproportionately rapid rate, indicating the entrance of some additional factor—probably the direct influence of the high temperature upon the egg-structure, as already indicated. The rate of activation under the influence of heat alone also increases very rapidly with rise in temperature ($Q_{10} = 200 - 400$).[2] Physical changes induced by heat in protein-containing systems—*e. g.*, heat-coagulation or the melting of gelatine gels—show similarly high temperature-coefficients;[3] so that it seems probable that temperatures of the activating range (30° to 38°) produce their effects by altering the physical condition of the structural colloids in the egg-system, and that this change secondarily facilitates or renders possible the chemical interaction upon which activation depends. In order to account for the above rise in the Q_{10} values at temperatures above 20° it seems necessary to assume that as the temperature approaches the region of heat-activation proper, the physical conditions become progressively more favorable for the interaction of butyric acid with the egg-component—*e. g.*, the resistance to the penetration of acid is lessened—so that the reaction is accelerated at a rate higher than can be explained by the influence of temperature upon reaction-velocity alone.

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[1] *Loc. cit.*

A large number of experiments of the above kind were performed between May 30 and June 28, 1916. During this period at Woods Hole starfish eggs are abundant, and more uniform in their behavior than later in the season. The general character of the results following simple exposure to butyric acid solutions for varying periods has already been described in my two preceding papers; detailed descriptions of separate series are therefore unnecessary, and the essential results can be presented most concisely in the form of tables. Table I summarizes the results of ten typical series of experiments at temperatures of 8° to 28°. The experiments are divided into two groups, *A* and *B*, one carried out early in June, the other two weeks later. My experience of the last three summers has shown that the effective durations of exposure, both to warm sea-water and to butyric acid solution, are decidedly longer early in the breeding season (up to the middle of June) than later. This is illustrated by the two series of experiments at 18° in Table I.; thus on June 10 the optimum exposure was about 14 minutes; two weeks later (June 24) it had fallen to about 6 minutes.[1] The physiological condition of the eggs used in each group may be regarded as approximately uniform, although certain individual differences of susceptibility are apparent. In both groups the condition of the eggs was good throughout, practically all eggs in the control dishes undergoing normal maturation and developing to larval stages after fertilization; but in the later group the physiologically equivalent exposures are on the average only about half as long as in the earlier. Experiments performed during the intervening period (June 13 to 21) show intermediate conditions (see Table IV).

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[1] Greeley (BIOLOGICAL BULLETIN, 1903, Vol. 4, p. 129) found with starfish eggs exposed to a mixture of 5 c.c. *n*/10 HCl *plus* 100 c.c. sea-water an approximate optimum at 23° of 5 minutes, at 11° of 15–30 minutes, and at 2° of 45–60 minutes. This indicates for the action of HCl a temperature-coefficient of a similar order. Loeb and Hagedoorn found a similar coefficient for the production of fertilization-membranes in sea-urchin eggs by butyric acid, the physiologically equivalent exposures being twice as long at 10° as at 20° ("Artificial Parthenogenesis and Fertilization," p. 146). The action of hypertonic sea-water shows a similar temperature-coefficient (*cf.* Greeley, *loc. cit.*, pp. 131–133; Loeb, *loc. cit.*, p. 102).

[2] R. S. Lillie, BIOLOGICAL BULLETIN, *loc. cit.*

[3] *Cf.* Schroeder, *Zeitschr. f. physik. Chem.*, 1903, Vol. 45, p. 75; Levites, *Kolloid-Zeitschrift*, 1907, Vol. 2, p. 211; Freundlich, "Kapillarchemie," 1909, p. 416; Chick and Martin, *Journal of Physiology*, 1910, Vol. 40, p. 404, and 1912, Vol. 45, p. 261.

The estimates of temperature-coefficients for a given temperature-interval (*e. g.*, 8° to 10°) were always made from results obtained with a single lot of eggs; these eggs in nearly all cases came from a single animal. The eggs used in determining each temperature-coefficient thus form a homogeneous group. The total number of series of experiments, each with a separate lot of eggs, performed during the whole period of May 30 to June 28 was twenty-three. Table IV at the end of the paper gives a summary of the character and essential results of all of these experiments.

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[1] Compare the optima for the same concentrations of butyric acid given in Table III (June 7–13, 1915) and Table V (July and August, 1915) in my article in *Journ. Biol. Chem.*, *loc. cit.*, pp. 243 and 246.

TABLE I.

A. EXPERIMENTS PERFORMED IN EARLY JUNE.

Lot 2. June 9; 8° and 18°. One starfish. Controls normal.

Temp. of Sol.	Times of Exposure and Percentages of Eggs Forming Blastulæ.												
	3 M.	6 M.	9 M.	12 M.	15 M.	18 M.	22 M.	26 M.	30 M.	35 M.	40 M.	46 M.	54 M.
8°	0	0	0	0	0	0	*ca.* 1%	35–40%	70–80%	85–90%	*ca.* 90%	60–70%	10–20%
18°	0	65–70%	95%	*ca.* 90%	20–30%	0	0	0	—	—	—	—	—

Lot 3. June 9; 10° and 12°. One starfish. Controls normal.

Temp.	3 and 6 M.	9 M.	12 M.	15 M.	18 M.	22 M.	26 M.	30 M.	35 M.
10°	0	0	0	10–15%	40–50%	85–90%	95%	*ca.* 95%	*ca.* 90%
12°	0	<1%	25–35%	80–90%	*ca.* 95%	>95%	>95%	>90%	20–30%

Lot 4. June 10; 14°, 16°, 18°. One starfish. Controls normal.

Temp.	2 M.	4 M.	6 M.	8 M.	10 M.	12 M.	14 M.	16 M.	18 M.	20 M.	22 M.	24 M.	26 M.
14°	0	0	0	0	<1%	10–15%	10–15%	25–30%	40–50%	50–60%	70–80%	70–80%	*ca.* 70%
16°	0	0	0	*ca.* 1%	*ca.* 5%	25–35%	50–60%	70–80%	75–85%	75–85%	70–80%	—	—
18°	0	0	*ca.* 1%	25–35%	50–60%	70–80%	80–90%	80–90%	25–35%	—	—	—	—

Lot 6. June 12; 8° and 10°. Two starfish. Controls normal.

Temp.	4 M.	8 M.	12 M.	16 M.	20 M.	24 M.	28 M.	32 M.	36 M.	40 M.	44 M.	48 M.
8°	0	0	0	*ca.* 1%	*ca.* 2–3%	5–10%	40–50%	70–80%	80–90%	80–90%	65–75%	5–10%
10°	0	0	*ca.* 1%	10–15%	30–40%	60–70%	80–90%	85–90%	85–90%	70–80%	50–60%	10–15%

Lot 7. June 12; 20°, 22°, 24°. Two starfish. Controls normal.

Temp.	1 M.	2 M.	3 M.	4 M.	5 M.	6 M.	7 M.	8 M.	9 M.	10 M.
20°	0	0	<1%	*ca.* 5%	25–30%	*ca.* 50%	60–70%	*ca.* 90%	*ca.* 95%	*ca.* 90%
22°	0	0	*ca.* 10%	30–40%	70–80%	*ca.* 95%	*ca.* 95%	*ca.* 90%	—	—
24°	1%	30–35%	*ca.* 90%	*ca.* 95%	40–50%	10–15%	—	—	—	—

B. EXPERIMENTS PERFORMED IN LATE JUNE.

Lot 23. June 26; 8° and 10°. One starfish. Good control (fair number of eggs fail to maturate).

Temp.	3 M.	6 M.	9 M.	12 M.	15 M.	18 M.	21 M.	24 M.	27 M.	30 M.	33 M.	36 M.
8°	0	0	<1%	5–10%	20–30%	30–40%	*ca.* 50%	50–60%	30–40%	5–10%	<1%	0
10°	pr. 0	<1%	*ca.* 1%	5–10%	55–60%	65–75%	65–75%	25–35%	15–20%	10–15%	——	—

Lot 22. June 26; 12°, 14°, 16°. One starfish. Controls normal.

Temp.	2 M.	4 M.	6 M.	8 M.	10 M.	12 M.	14 M.	16 M.	18 M.	20 M.	22 M.
12°	*ca.* 5%	15–20%	25–35%	80–90%	70–80%	70–80%	*ca.* 75%	50–60%	40–50%	*ca.* 10%	0
14°	10–15%	*ca.* 50%	60–70%	60–70%	80–85%	*ca.* 50%	*ca.* 10%	*ca.* 1%	0	0	—
16°	30–35%	*ca.* 50%	80–85%	70–80%	15–20%	2–3%	0	0	0	——	—

Lot 21. June 24; 18°, 20°, 22°, 24°. One starfish. Controls normal.

Temp.	½ M.	1 M.	1½ M.	2 M.	2½ M.	3 M.	3½ M.	4 M.	5 M.	6 M.	7 M.	8 M.	9 M.
18°	—	0	—	<1%	—	15–20%	—	60–70%	80–85%	80–90%	70–80%	60–70%	25–35%
20°	—	0	—	15–20%	—	*ca.* 95%	—	*ca.* 90%	80–90%	35–45%	*ca.* 1%	0	
22°	0	0	35–40%	75–85%	75–85%	80–90%	60–70%	—	—	—	—	—	—
24°	0	5–10%	*ca.* 90%	*ca.* 90%	50–60%	<1%	—	—	—	—	—	—	—

Lot 17. June 22; 24°, 26°. One starfish. Controls normal.

Temp.	½ M.	1 M.	1½ M.	2 M.	2½ M.	3 M.
24°	0	25–35%	80–90%	95%	40–50%	10–15%
26°	<1%	70–80%	70–80%	10–15%	0	0

Lot 20. June 24. 26°, 28°. One starfish. Controls normal.

Temp.	½ M.	1 M.	1½ M.	2 M.
26°	5–10%	80–90%	30–35%	0
28°	80–90%	pr. 0	0	0

In Table I the figures represent the approximate percentages of eggs forming free-swimming blastulæ with each exposure. The estimates of percentages were made in watch-glasses under a low power; a large number of eggs were used in making each estimate, collected at random from the bottom and sides of the dish. The probable error of such estimates is assumed to be about 5 per cent.; hence the values are given as approximations; this procedure appears to be as accurate as the conditions permit. There is always a clearly defined optimum in such a series; but often two and sometimes three successive dishes near the optimal region show equally good conditions, especially if the intervals between successive exposures are brief. In such cases the optimum used in calculating temperature coefficients is the arithmetical mean of the favorable exposures. For convenience in supervision the optima are printed in heavy type.

Table II summarizes the results of the above experiments, and gives the temperature-coefficients for each series. The coefficients are given as the ratios between the velocities at two temperatures 10° part (Q_{10} values), in accordance with the usage customary in physiological literature. It is assumed that the velocity increases through the entire interval of 10° at the same proportional rate as through the observed interval of 2°; the value of Q_{10} is then $(T_n/T_{n+2})^5$, T_n and T_{n+2} being the observed optimal times of exposure at the two temperatures.[1] Each value of Q_{10} is derived from observations made upon the same lot of eggs at the two temperatures.

In each group the temperature-coefficient is seen to increase steadily as the temperature rises. The two groups are best compared if the Q_{10} values are placed side by side as in Table III.

Further experimental data are given in Table IV at the end of the paper, which summarizes the results of all of last season's experiments. In this table the experiments for each temperature are grouped together; the decrease in the effective times of ex-

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[1] The temperature-coefficient as thus expressed is obtained from any pair of observations by the formula,

$$Q_{10} = \left(\frac{V_{t_1}}{V_{t_2}}\right)^{\frac{10}{t_1 - t_2}}$$

V_{t_1} and V_{t_2} being the velocities observed at the two temperatures t_1 and t_2.

TABLE II.

GROUP *A*. (June 9–12).

Number and Date of Series.	Temperatures of Solution and Optimum Exposures.	Mean Optimum.	Value of Q_{10}.
2. June 9	8°:35–40 min.	37.5 m.	3.55
	18°: 9–12 min.	10.5 m.	
6. June 12	8°:36–40 min.	38 m.	1.75
	10°:32–36 min.	34 m.	
3. June 9	10°:26–30 min.	28 m.	2.2
	12°:22–26 min.	24 m.	
4. June 10	14°: 22–24 min.	23 m.	2.6
	16°: 18–20 min.	19 m.	4.65
	18°: *ca.* 14 min.	14 m.	
7. June 12	20°: 9 min.	9 m.	5.0
	22°: 6–7 min.	6.5 m.	11.6
	24°: 4 min.	4 m.	

GROUP *B*. (JUNE 22–26).

23. June 26	8°: 21 –24 min.	22.5 m.	2.0
	10°: 18 –21 min.	19.5 m.	
22. June 26	12°: 8 –14 min.	11 m.	2.7
	14°: 8 –10 min.	9 m.	3.55
	16°: 6 – 8 min.	7 m.	
21. June 24	18°: 5 – 6 min.	5.5 m.	5.0
	20°: 3 – 5 min.	4 m.	6.4
	22°: 2½– 3 min.	2.75 m.	9.5
	24°: 1½– 2 min.	1.75 m.	
17. June 22	24°: 2 min.	2 m.	10.5
	26°: 1 – 1½ min.	1.25 m.	
20. June 24	26°: *ca.* 1 min.	1 m.	32.0
	28°: *ca.* ½ min.	.5 m.	

TABLE III.

Interval.	Values of Q_{10}. *A*. June 9–12.	*B*. June 22–26.
8°–10°	1.75	2.0
10°–12°	2.2	
12°–14°		2.7
14°–16°	2.6	3.55
16°–18°	4.65	
18°–20°		5.0
20°–22°	5.0	6.4
22°–24°	11.6	9.5
24°–26°		10.5
26°–28°		32.

posure with rise of temperature, and the tendency to a shortening of the reaction-time as the season advances, are both shown clearly. The date of each experiment is given, and each lot of eggs is designated by number so as to render possible comparison between different lots. It will be noted that in general the temperature-coefficients between 8° and 18° are of the usual order of chemical reaction-velocities, but that above 18° they increase rapidly. The significance of this increase has already been considered.

(*b*) *Experiments at Temperatures Below 8°.*

In these experiments the sea-water and solutions used were first cooled at the required temperature, and then added to the beakers containing the eggs as before. The temperatures were kept constant during the period of the experiment by immersing the beakers in battery-jars containing cold water, together with a sufficient quantity of chopped ice to keep the temperature at the desired point. With proper care the fluctuations of temperature are slight under these conditions—usually less than a degree on either side of the temperature chosen, an approximation sufficient for the purpose of these experiments.

Table V gives the results of three series of experiments with a single lot of eggs at 2°, 4°, and 6°. The eggs were placed, shortly before the separation of the first polar body, in the solution of butyric acid (.006 *n* in sea-water) at the three temperatures, and portions were returned to normal sea-water at room temperature after exposures varying from 5–65 minutes.

Table V.

June 13, 1916. Eggs from Two Starfish. Controls Show Normal Maturation and Normal Development after Fertilization.

Temperature.	Times of Exposure and Percentage of Eggs Forming Blastulæ.										
	5 M.	10 M.	15 M.	20 M.	25 M.	30 M.	35 M.	40 M.	45 M.	50 M.	55 and 60 M.
Ser. *A*, 2°..	*ca.* 1%	2–3%	1–2%	3–4%	15–20%	35–40%	25–35%	0	0	0	0
Ser. *B*, 4°..	<1%	*ca.* 5%	5–10%	15–20%	35–40%	55–60%	20–25%	0	0	0	0
Ser. *C*, 6°..	3–4%	1–2%	*ca.* 5%	15–20%	30–40%	*ca.* 90%	30–40%	*ca.* 5%	*ca.* 1%	0	0

With exposures of 35 minutes and longer a large proportion of the eggs undergo complete cytolysis ("ghosts" next day), especially in Series *A* ($A > B > C$).

In each series the eggs show an optimum of activation with an exposure of about 30 minutes. The differences between the three series are not great; in each series over-exposure (35 minutes and longer) is followed by the complete breakdown and disintegration of a large proportion of eggs; by next day nothing is left of these eggs but a thin structureless residue ("ghosts"). This dissolution is much more complete than that resulting from moderate over-exposure to butyric acid at higher temperatures. It will be noted that the proportion of eggs developing favorably with the optimum exposure is highest at 6° and lowest at 2°; *i. e.*, the destructive effect is greater the lower the temperature.

The higher rate of activation in these experiments, as compared with that observed at slightly higher temperatures (8° to 10°), is almost undoubtedly the expression of a summation of the two separate effects of cold and butyric acid. This is indicated by the results of exposure to normal sea-water at these temperatures. On June 14 eggs were exposed, under the same conditions as above, to normal sea-water at 2°, 4°, and 6°. The result was a typical though partial activation in each series; a large majority of eggs—not all—formed membranes and underwent irregular fragmentation and breakdown without further development. In each series a considerable proportion of eggs (about one third to one fourth) remained apparently unaffected, and later underwent the usual postmaturational coagulation without membrane-formation or development. Several other similar experiments yielded similar results. The above durations of exposure are evidently too brief for complete activation. With longer exposures (about 6 hours) a few eggs may form larvæ under these or similar conditions.[1] Evidently low temperature alone may initiate the activation-process; hence with cold and butyric acid combined the rate of activation may be more rapid than with butyric acid at the higher temperatures of 8° and 10°, which by themselves have no activating effect upon these eggs. Eggs exposed on June 15 to sea-water at 8° and 10° for periods ranging from 5 to 50 minutes showed no signs of activation.

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[1] In Greeley's experiments (*loc. cit.*, 1902) the best results were obtained when the eggs were exposed, beginning three to four hours after the completion of maturation, for six to nine hours to temperatures of 4°–5°. The highest proportion of larvæ obtained was 20 per cent.; usually only 1–2 per cent. of eggs formed larvæ. The period after maturation is completed is in general unfavorable for activation in starfish eggs.

The rate of activation under the influence of low temperature alone is very gradual. Out of eight additional series of experiments with cold normal sea-water (1° to 5°), performed at different times up to July 19, and in which the longest exposures were respectively $4\frac{1}{2}$, 8, 6, $7\frac{3}{4}$, 8, 7, 7, and 6 hours, only one yielded any swimming larvæ; in this series (July 2) about one per cent. of the eggs formed blastulæ after exposures to sea-water at 1° to 3° for 7 hours (see Table VI). In the other series the only evident effect was the formation of fertilization-membranes in a variable proportion of eggs, followed by irregular changes of form and breakdown; with the longer exposures a certain proportion of eggs cleaved in some experiments, but none developed further.

It had previously been observed that the addition of alcohol caused a decided acceleration of the activation-process in butyric acid solution or warm sea-water. For example, in sea-water containing 3 or 4 volumes per cent. C_2H_5OH the exposure to .003 *n* butyric acid required to cause a majority of eggs to form blastulæ was shortened from 7 or 8 minutes to about 3 minutes.[1] This result suggested the possibility that the rate of activation at low temperatures might similarly be increased by the presence of alcohol. The experiments described in Table VI show that this is the case.

It is clear that activation at low temperatures as well as at high temperatures is favored by the presence of alcohol. In solutions of the above concentrations, alcohol, acting alone at ordinary temperatures, has no evident effect; eggs exposed on June 29 to 4 vols. per cent. alcohol at the three temperatures 18°, 22°, and 25°, for periods ranging from 2 to 24 minutes, showed neither membrane-formation nor development. The favorable effect in the above series is thus due not to the direct activating influence of the alcohol, but to a facilitation or acceleration of the activation-reaction, which takes place spontaneously though slowly at these low temperatures. It seems probable that the alcohol alters the colloidal substratum in such a manner as to increase the diffusion-rates of the interacting substances; at least some change in the structural or catalytic conditions determining reaction-velocities is indicated. The terms facilitation and sensitization merely describe or classify the effect without throwing any further light upon its physico-chemical nature.

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[1] R. S. Lillie, *Jour. Biol. Chem., loc. cit.*, p. 246, footnote.

TABLE VI.

July 1. Eggs from one starfish were placed in (*A*) cold normal sea-water, and (*B* and *C*) cold sea-water containing respectively 3 and 4 vols. per cent. ethyl alcohol. The eggs were kept in flasks immersed in an ice-water bath; the temperature of each flask was about 3° (varying between 2° and 4°). At intervals of 2, 4, 6, and 8 hours eggs were transferred to sea-water at room temperature.

The majority of control eggs (*i. e.*, unfertilized and sperm-fertilized eggs at room temperature) showed normal behavior. Results were as follows:

Times of Exposure.	*A.* Normal Sea-water.	*B.* 3 V. % C_2H_5OH.	*C.* 4 V. % C_2H_5OH.
1. 2 h.	Most eggs form membranes and break down.	All eggs form membranes; 4 or 5 blastulæ found.	All form membranes; a few blastulæ.
2. 4 h.	Same as *A* 1; no blastulæ.	A few blastulæ.	*Ca.* 4–5.% of eggs form blastulæ.
3. 6 h.	No blastulæ.	A few blastulæ.	*Ca.* 15–20% blastulæ.
4. 8 h.	No blastulæ; some cleavages.	Blastulæ more numerous; *ca.* 1–2%.	*Ca.* 30–35% blastulæ.

The results of a similar experiment on July 2, with 4 and 5 vols. per cent. alcohol, were as follows:

Exposures.	*A.* Normal Sea-water.	*B.* 4 V. % C_2H_5OH.	*C.* 5 V. % C_2H_5OH.
1. 1 h.	20–25% of mature eggs form membranes; one blastula found.	All mature eggs form membranes; 10 to 15% blastulæ formed.	Membranes in all mature eggs; *Ca.* 10–15% blastulæ.
2. 3½ h.	Like A1; a few blastulæ.	*Ca.* 25–35% blastulæ.	*Ca.* 35–45% blastulæ.
3. 4½ h.	Like *A* 2.	*Ca.* 30–40% blastulæ.	*Ca.* 70–80% blastulæ.
4. 7 h.	Blastulæ more numerous; *ca.* 1%.	*Ca.* 10–15% blastulæ.	*Ca.* 30–40% blastulæ.

The essential question is why low temperatures should so alter the conditions within the cell as to render possible the activation-reaction. That cold may cause definite structural changes in many cells, leading to cytolysis or other characteristic effects, is well known. Such effects are often found at temperatures well above the freezing-point, *e. g.*, the fatal action of sub-normal temperatures upon tropical marine animals[1] or warm-blooded vertebrates. Greeley describes protoplasmic condensation and loss of water in *Stentor* and other organisms as a result of prolonged exposure to low temperatures (6° and lower in most of his experiments), and he calls attention to various resemblances between the effects produced by cold and by hypertonic solutions.[2] It does not seem probable, in the light of more recent knowledge, that the activation following the exposure of starfish eggs to cold and to hypertonic sea-water respectively can be referred directly to the same cause, namely, loss of water, as Greeley supposed; but the evidence that a lowering of temperature below a certain critical point produces definite structural alterations has an obvious bearing on the present problem. In the case of high temperatures (above 30°) the activating influence is probably to be referred to structural changes in the protoplasmic system, as the temperature-coefficients indicate; and the same appears to be true for cold. Changes in the physical condition of the structural colloids—*e. g.*, gelation, dehydration, altered aggregation-state—may alter locally the permeability or other properties of the protoplasmic system (*e. g.*, of membranes or other barriers to diffusion), and thus render possible interactions which are not possible at ordinary temperatures. This seems to be the most consistent general explanation for the fact that in the starfish egg temperatures both above and below a certain range, 8° to 28°, may induce activation. This range may be regarded as corresponding to the range of stability of the structures concerned.

General Discussion.

The phenomena under consideration in the present paper exhibit many features in common with those accompanying or conditioning cytolysis in other cells. Cytolysis in weak solutions of acids and bases, as well as under the influence of high temperatures, is a phenomenon of general occurrence, and its conditions resemble closely those already described as determining the rate and character of activation in starfish eggs. Both processes require time, and end in structural alterations of a definite kind; in the egg membrane-formation is the first visible change, which may be followed by development; in cytolysis the essential effect appears to be an alteration of the surface-layer or plasma-membrane of the cell; this structure loses its normal insulating or semi-permeable properties, with the result that the diffusible cell-constituents pass into solution in the surrounding medium; the cell then disintegrates. The physico-chemical conditions of cytolysis have been investigated most completely in red blood-corpuscles, and exact data are available with reference to the influence of both the concentration of the hæmolytic agent and of temperature upon the rate of hæmolytic action. Many facts indicate that a change similar in kind to that underlying cytolysis, only reversible and of brief duration, forms a primary feature of both normal and artificial activation. According to Loeb an incipient or superficial cytolysis is the first stage in the chemical activation of sea-urchin eggs;[1] and this view has been substantiated by a large number of investigations, which have shown that cytolytic agents of the most varied kind, chemical and physical, may cause parthenogenesis.

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[1] The tropical medusa Cassiopea shows an interesting parallel to the conditions in starfish eggs. The animals may be cooled to 9.5° and recover, if immediately returned to sea-water at the normal temperature of 29°; but if cooled to 7° or 8° there is no recovery; some irreversible change is produced and the tissues disintegrate on return to warm water (E. N. Harvey, "Effect of Different Temperatures on *Cassiopea*," Carnegie Institution Publications, 1910, No. 132, p. 32).

[2] Greeley, *Amer. Journ. Physiol.*, 1902, Vol. 6, p. 122; Biological Bulletin, 1903, Vol. 5, p. 42.

All of this evidence indicates that the initial change in the activation of the resting egg is *superficial*, and associated with a general increase in the permeability of the egg-surface to water-soluble substances and water.[2] One general consequence of such a change is electrical depolarization; and from the analogy with the phenomena of stimulation it seems probable that this electrical variation, as such, forms the critical change of condition by which the course of the metabolic processes in the egg is so modified as to initiate development. Structural and metabolic alterations go hand in hand and mutually influence each other, as has lately been especially emphasized by Child;[3] and the initiation of development in unfertilized eggs through a slight change in the physical state of the surface-film offers perhaps the clearest illustration of this principle. The general conditions of cytolysis have thus an intimate bearing upon the question of the nature of the initial process in activation.

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[1] J. Loeb. "Artificial Parthenogenesis and Fertilization," Chapter 17.

[2] *Cf.* my recent paper in *Amer. Journ. Physiol.*, 1916, Vol. 40, p. 249.

[3] C. M. Child, "The Regulatory Processes in Organisms," *Journal of Morphology*, 1911, Vol. 22, p. 173; *cf.* also "Senescence and Rejuvenescence," University of Chicago Press, 1915, especially Chapters 1 and 2.

Arrhenius and Madsen have studied by exact methods the conditions of hæmolysis in weak solutions of acids and bases.[1] Their experiments with ammonia offer perhaps the closest analogies to the above experiments with starfish eggs. The rate of hæmolysis has been found to be directly proportional to the concentration of ammonia, and to increase at a rapid rate with rise of temperature. They conclude that the hæmolytic effect depends upon a chemical reaction of the monomolecular class. Evidently this reaction must proceed to a certain stage before the corpuscles are sufficiently altered to release their hæmoglobin. The times required to produce at constant temperature (0°) a definite degree of hæmolysis by different concentrations of ammonia are given in the following table.[2]

TABLE VII.

Concentration of NH_3 (n).	Time of Action Required for a Given Per Cent. of Haemolysis (in Minutes).			
	10 %.	20 %.	30 %.	40 %.
.001 n	26 m.	35 m.	44 m.	53 m.
.00227	10 m.	15 m.	18 m.	23 m.
.00435	5.5 m.	9 m.	12 m.	14 m.
.0075	....	4 m.	6.2 m.	8 m.

The numbers in the vertical columns show that the rate of hæmolytic action is directly proportional to the concentration of ammonia (qt = const.). In the horizontal columns the relations between time of action and degree of hæmolysis are shown for each concentration of ammonia; these relations are also similar to those between time of exposure and degree of activation in starfish eggs; for example, in the experiment of June 10 at 14° (Table I) the proportion of fully activated eggs with exposures of 14, 16, 18, and 20 minutes were respectively

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[1] *Cf.* Arrhenius, "Immunochemistry," Macmillans, 1907, Chapter 4; also "Quantitative Laws in Biological Chemistry," London, Bell and Co., 1915.

[2] "Immunochemistry," p. 101; "Quantitative Laws," p. 64.

10–15 per cent., 25–30 per cent., 40–50 per cent., and 50–60 per cent.; compare (*e. g.*) the proportion of corpuscles hæmolyzed by .00227 *n* NH_3 in 10, 15, 18 and 23 minutes, viz., respectively 10, 20, 30, and 40 per cent. The influence of temperature upon the rates of the two processes is also of the same general kind, although certain differences are seen; in both cases the average value of the temperature-coefficients is greater than that of typical chemical reaction-velocities; but in hæmolysis the variability of the coefficient with change of temperature is less, and its value decreases with increase in the effective time of action.[2] The agreements, however, are sufficiently close to indicate that the same kind of process lies at the basis of both effects,—consisting apparently in the progressive combination of the acid or base with some cell-constituent until a certain critical quantity or local concentration of reaction-product is formed. Arrhenius also infers a chemical binding of the hæmolyzing acid or base to some substance in the erythrocytes.

In general we may conclude that in both cytolysis and the activation of the egg-cell the characteristic effect depends primarily upon the formation and accumulation of a reaction-product whose presence is the cause or condition of some definite physical change of state or structural alteration in the cell-system, especially in the surface-layer. In the egg-cell the structural conditions under which the normal metabolic reactions take place are modified, and the course of metabolism is changed; development then proceeds. In either type of cell, if an insufficient quantity of the activating substance is formed—as by too brief exposure—the effect is incomplete or fails to appear; thus in order to liberate hæmoglobin from erythrocytes by means of a given solution of ammonia at a constant temperature, a certain minimal time of exposure is required; with briefer exposures no visible effect is produced. We have seen that in the starfish egg exposures briefer than the optimum may produce visible effects (membrane-formation, imperfect cleavage, etc.) indicating a partial degree of activation; in the case of hæmolysis it is also to be assumed that brief exposures cause a partial cytolytic effect, which however is insufficient to liberate hæmoglobin and hence escapes observation. The egg differs from the erythrocyte in showing definite evidence of an incomplete reaction, but in other respects the conditions in the two kinds of cell are alike.

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[2] "Immunochemistry," pp. 107 *seq.*; "Quantitative Laws," pp. 65 *seq.*

All of the conditions indicate that in activation as in cytolysis the primary reaction takes place at the cell-surface. This view is confirmed by the promptness with which the course of the activation-process in butyric acid solution can be arrested at any desired stage by a return to sea-water. It seems clear that combination of the acid with some surface-component is concerned; a difference of half a minute or less in the time of exposure to .006 *n* butyric acid, especially at higher temperatures (24° and over) may make all of the difference between incomplete and complete activation, or between activation and destruction of the egg. In its prompt initiation in the solution of acid and its equally prompt arrest by return to sea-water the activation-reaction resembles closely the type of response characteristic of sensory structures like taste-buds or other chemical receptors in normal chemical stimulation. Crozier has recently reached the conclusion that in this case also stimulation is the result of a chemical surface-interaction.[1] Here again the existence of a far-reaching parallelism between the process of stimulation and the activation of the egg-cell is indicated. It appears probable that advance in the general physiology of stimulation will furnish the key to the interpretation of the activation-process.

The high temperature-coefficients of heat-cytolysis and of heat-activation in eggs indicate clearly that alterations of certain structural colloids of the cell lie at the basis of these effects. Reactions are thus enabled to take place in the egg which in some manner lead to the initiation of development. The initial chemical reaction in heat-activation is probably simple in character and similar to that of acid-activation, as already suggested. Apparently this reaction forms the condition of some definite structural change in the egg-system; this change is followed by the series of developmental changes.

It is possible that an autolytic process may form the first step in the metabolic sequence conditioning development. This is suggested not only by the fact that autolysis typically leads to structural breakdown and cytolysis, but also and more especially by its being characteristically furthered by many of the general cytolytic and parthenogenetic agents, such as lipoid-solvents, acids and high temperatures. Ether, chloroform and alcohol greatly accelerate the autolysis of liver-cells and shorten the latent period of the process;[1] and acids, including CO_2, are well known to have the same effect.[2] Chiari attributes the effect of lipoid-solvents to an increase of protoplasmic permeability, allowing readier diffusion of enzymes and the other substances concerned. There is also evidence that autolytic processes underlie various structural and other physiological changes, some of which are of a kind frequently met with in development, *e. g.*, atrophic changes[3] (to which are related normal regressive processes like the involution of the uterus and the resorption of larval structures in metamorphosis), and the translocation of reserve materials; the utilization of tissue-proteins in starvation also appears to be dependent upon autolysis.[1]

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[1] *I. e.*, the time required for stimulation is much shorter than that required for visible penetration of the acid into the cell-interior; *cf.* W. J. Crozier, *Jour. Biol. Chem.*, 1916, Vol. 24, p. 255; *cf.* pp. 270 *seq.*; *Journal of Comparative Neurology*, 1916, Vol. 26, p. 1.

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[1] Chiari, *Arch. f. exper. Path. u. Pharm.*, 1908, Vol. 60, p. 256; *cf.* also Yoshimoto, *Zeitschr. f. physiol. Chem.*, 1908, Vol. 58, p. 341.

[2] *Cf.* Hedin and Rowland, *Zeitschr. f. physiol. Chem.*, 1901, Vol. 32, p. 241; Schryver, *Biochemical Journal*, 1906, Vol. 1, p. 123; Arinkin, *Zeitschr. f. physiol. Chem.*, 1907, Vol. 53, p. 192; Bellazi, *ibid.*, 1908, Vol. 57, p. 389; Yoshimoto, *ibid.*, 1908, Vol. 58, p. 341; Bradley, *cf.* footnote page 153. Bellazi and Yoshimoto describe experiments with CO_2. *Cf.* also Lacqueur, *Zeitschr. f. physiol. Chem.*, 1912, Vol. 79, p. 82; Lacqueur finds that carbon dioxide promotes and oxygen checks the autolysis of liver-cells. According to Lyon and Shackell (*Journal of Biological Chemistry*, 1909, Vol. 7, p. 371) acetic acid promotes the autolysis of sea-urchin eggs.

It should be noted that in the activation of eggs by acid the visible effects of the treatment do not appear at once, but only after the eggs have been returned to normal sea-water; this shows that for the initiation of the membrane-forming process *time* is required, representing possibly the latent period of the enzyme action. Hedin's observation that a *temporary* treatment of various tissues with acetic acid greatly increases the subsequent rate of autolysis—*i. e.*, the autolysis is decidedly more rapid in previously treated than in untreated portions of tissue under otherwise similar conditions, and even in presence of alkali—may have a bearing here (*Cf.* Hedin, *Festkrift O. Hammarsten*, Upsala, 1906; also Rhodin, *Zeitschr. f. physiol. Chem.*, 1911, Vol. 75, p. 197). Hedin ascribes this result to the destruction of an inhibitory substance by the acid. This suggests the possibility that in the activation of eggs by acid the deciding factor may be the destruction of an antibody (anti-protease?) in the egg-cortex; an activation so induced would be irreversible (as seems in fact to be the case). This view, however, does not seem consistent with the observed relations between concentration of acid and time of action, as described above (see footnote on page 132). The question as to the precise conditions of acid-activation is evidently an open one.

[3] *Cf.* Jacoby, *Zeitschr. f. physiol. Chem.*, 1900, Vol. 30, p. 174.

The recent studies of Bradley and his associates have thrown an interesting light upon the conditions under which acid promotes autolysis of liver-cells.[2] Apparently the acid does not act simply by rendering the reaction favorable to the activity of the cell-proteases, for increasing the acidity does not necessarily increase the hydrolysis of all proteins;[3] moreover certain foreign proteins like gelatine or peptone are readily digested in neutral or slightly alkaline solution in which the liver-proteins resist digestion. It would appear that the intracellular proteases are capable of activity under these conditions, provided they are furnished with the appropriate substrate. The essential action of the acid consists rather in altering the character of the normal proteins of the cell, and rendering these a favorable substrate for the proteases present. The autolytic process is thus activated by the addition of acid; the increase in the rate and extent of autolysis is roughly proportional to the amount of acid added.[1]

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[1] *Cf.* Lane-Claypon and Schryver, *Journal of Physiology*, 1904, Vol. 31, p. 169; Schryver, *Biochemical Journal*, *loc. cit.* In "Senescence and Rejuvenescence," Chapter 2, Child calls attention to the general importance of the process of reduction or regression during development and regeneration. Regarded in the purely chemical sense, regression is the reverse of construction; strictly speaking, both processes are constantly and simultaneously at work in any living organism; it is only when regression exceeds construction that the substance of the organism undergoes visible decrease. Local regressive changes of this kind appear always to accompany regeneration; the translocation of material from one part to another is a necessary condition of the process; and such evidence as we possess indicates that autolysis is an important factor in any local structural breakdown which furnishes the material required for construction elsewhere (*cf.* Schryver's observations on the increased rate of autolysis in the livers of fasting animals, *loc. cit.*). It must be admitted, however, that this evidence is deficient in many respects (*cf.* Morse, *Amer. Journ. of Physiol.*, 1914, Vol. 36, p. 145), and it seems likely that the essential factors in the disintegration as well as in the building up of structure are unknown at present. The possible rôle of autolysis in regeneration has been discussed recently by Loeb ("The Organism as a Whole from the Physicochemical Viewpoint," Putnams, 1916, p. 178); if autolysis is a factor in regeneration, we may safely assume that it is important in developmental processes in general, including the development of the organism from the egg, and in this case probably from the very first, as suggested in the hypothesis put forward in the text.

[2] Bradley and Morse, *Journal of Biological Chemistry*, 1915, Vol. 21, p. 209; Bradley, *ibid.*, 1915, Vol. 22, p. 113; Bradley and Taylor, *ibid.*, 1916, Vol. 25, pp. 261, 363.

[3] The liver proteins are very sensitive to increase of acidity (Bradley and Taylor, *loc. cit.*, 1916, p. 262), but foreign proteins added to the autolyzing mixture may or may not show increased digestion under these conditions; *e.g.*, the digestion of egg albumin is greatly increased by acid, while that of casein and peptone remains unaltered; $MnCl_2$ promotes the digestion of liver-proteins, but not that of egg-albumin.

If this interpretation is applied to the case of the activation of the starfish egg by butyric acid, certain interesting possibilities appear. The acid appears to act by combining chemically, as already seen. If we assume that it forms a combination with some structure-forming protein, which then becomes hydrolyzable by some enzyme present, the above facts appear in a clearer light. The transformation of a certain definite quantity of this protein into substrate for the enzyme would then form the condition for complete activation. The rate of this transformation would correspond to the rate of the activation-process and would be proportional to the concentration of acid. We suggest, therefore, that acids cause activation by forming a hydrolyzable combination for some intracellular enzyme, probably one of well-defined specificity. The activating influence of high temperatures also becomes intelligible on this hypothesis, and may be referred to the production of an acid (*e. g.*, lactic) which then combines to form the hydrolyzable substrate; the similarity in the time-relations of heat-activation and acid-activation is thus explained. The autolysis following this combination would constitute the first step in development.

What is most significant from the standpoint of the general physiology of development is the evidence that a structural modification, in itself apparently simple, is sufficient to alter the whole course of egg-metabolism in a definite and predetermined manner. We must recognize that development is essentially the expression or outcome of a complex chain or nexus of metabolic processes, which is released by the activating reaction. Evidently these processes include chemical interactions and syntheses of a highly diversified and specific kind; these latter determine the specific character of the development in any instance. Regarding their precise nature we are almost completely ignorant at present. But however complex and unanalyzable the whole developmental sequence may appear, its initiation is almost certainly dependent upon some slight and non-specific and primarily structural alteration in the egg-system.

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[1] Bradley and Taylor, *loc. cit.*, 1916, p. 274.

In the orderly progress of development from one stage to the next we must similarly assume at every stage a similar interdependence between the existing structural conditions and the rate and character of the form-determining as well as other metabolism. Each step in advance is thus largely a consequence of the immediately preceding structural modification, and in its own turn furnishes the structural conditions determining the next step. From this point of view the unification of the whole developmental sequence would seem to be conditioned, at least in its main features, by specific *structural* peculiarities, represented at the beginning of development by the inherited organization of the germ. This determines at the start—assuming normal external conditions—the course of the ensuing transformations, and hence the special kind of structure arising at each successive stage of development. Structure, however, is not alone to be considered; the germ is a metabolizing system, and the soluble and diffusible (non-structural) materials formed in metabolism undoubtedly also play an essential part, probably chiefly of a controlling or regulative kind (*e. g.*, as hormones). And since throughout development there is always this reciprocal interdependence between structure and metabolism, it is clear that such materials—which presumably change continually in their character as development proceeds—must influence at every stage the nature of the metabolic transformations and hence of the organized structure which is being built up. It seems further probable that the destruction or removal of inhibiting or injurious or anticatalytic compounds, as well as the formation and activation of specific catalysts (enzymes), is concerned in the developmental process from the very first.[1] In brief, the metabolism of the developing germ must be regarded as differing not qualitatively but only in certain of its quantitative aspects—especially in the predominance of growth and formative processes—from that of the adult organism.

[1] This is indicated (*e.g.*) by the existence in the sea-urchin egg of an antibody for the sperm-agglutinin or "fertilizin" (F. R. Lillie, *Journal of Experimental Zoölogy*, 1914, Vol. 16, p. 544).

As Child has expressed it, one chief problem in the physiology of development is to determine how metabolism produces structure.[1] The converse problem of how structure influences metabolism is equally important. The course of structural modification as development proceeds is orderly; to this must correspond a similarly orderly alteration in the character of the chemical transformations which furnish at the same time both the energy and the material for development. Another consideration is important here. Corresponding to the increasing structural diversity with advance in development there is an increasing chemical diversity; of this we have already ample evidence; hence the need for special mechanisms of integration and coördination, both structural and chemical, must become greater with each advance in development. Presumably the development of these mechanisms runs parallel with the development of the organism as a whole. The continued and stable existence of the latter at any stage in its life-history—of the embryo as well as of the adult—is in fact contingent upon the uninterrupted and adequate working of these regulatory processes. In this respect also the developing germ differs in no essential manner from the adult organism. The physiological problems presented by the organism at different developmental stages differ in degree rather than in kind. From the egg to the embryo and from the embryo to the adult the transitions are continuous; the physiology of maintenance and the physiology of growth and inheritance are at bottom the same.

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[1] *Journal of Morphology, loc. cit.*, p. 193.

TABLE IV.

OPTIMUM TIMES OF EXPOSURE TO .006 *n* BUTYRIC ACID IN SEA-WATER AT DIFFERENT TEMPERATURES.

Temperature.	Number of Lot and Date.	Range of Exposures.	Optimum Exposures with Results.	Exposures Next Above and Next Below Optimum with Results (% Blastulæ).
		Minutes	*Min. % blastulæ*	
8°	2, June 9	3–54	35–40 (85–90)	30 m. (70–80); 46 m. (60–70)
	6, “ 12	4–48	36–40 (85–90)	32 m. (70–80); 44 m. (65–75)
	9, “ 16	3–45	30–40 (?)	27 m. (?); 45 m. (?)
	15, “ 20	3–36	21–27 (20–35)	18 m. (15–20); 30 m. (few)
	23, “ 26	3–36	21–24 (50–60)	18 m. (30–40); 27 m. (30–40)
10°	3, June 9	3–35	26–30 (*ca.* 95)	22 m. (80–90); 35 *m.* (*ca.* 90)
	6, “ 12	4–48	28–36 (80–90)	24 m. (60–70); 40 m. (70–80)
	9, “ 16	3–36	24–36 (*ca.* 50)	21 m. (40–50);
	15, “ 20	3–33	*ca.* 18 m (40–50)	15 m. (35–45); 21 m. (35–45)
	23, “ 26	3–30	18–21 (65–75)	15 m. (55–60); 24 m. (25–35)
12°	2, June 9	3–35	18–26 (*ca.* 95)	15 m. (80–90); 30 m. (*ca.* 90)
	9, “ 16	3–36	21–24 (50–60)	18 m. (40–50); 27 m. (*ca.* 50)
	15, “ 20	3–30	12–15 (50–60)	9 m. (10–15); 18 m. (20–30)
	22, “ 26	2–22	8–12 (70–90)	6 m. (25–35); 14 m. (*ca.* 75)
14°	4, June 10	2–26	22–24 (70–80)	20 m. (50–60); 26 m. (*ca.* 70)
	10, “ 17	1–12	>12 m. (optimum not reached)	12 m. (15–20);
	12, “ 19	2–22	*ca.* 14 m. (65–70)	12 m. (30–40); 16 m. (40–50)
	14, “ 20	2–22	*ca.* 14 m. (70–80)	12 m. (65–75); 16 m. (*ca.* 50)
	22, “ 26	2–20	*ca.* 10 m. (80–85)	8 m. (60–70); 12 m. (*ca.* 50)
16°	4, June 10	2–22	18–20 (75–85)	16 m. (70–80); 22 m. (70–80)
	10, “ 17	1–12	>12 (optimum not reached)	12 m. (60–70)
	12, “ 19	2–18	*ca.* 10 (85–90)	8 m. (45–50); 12 m. (70–80)
	14, “ 20	2–18	*ca.* 10 (85–90)	8 m. (35–40); 12 m. (65–70)
	22, “ 26	2–18	6–8 (70–85)	4 m. (*ca.* 50); 10 m. (15–20)
18°	1, June 8	2–24	10–12 (80–90)	8 m. (65–75); 14 m. (60–70)
	2, “ 9	3–26	9–12 (90–95)	6 m. (65–70); 15 m. (20–30)
	4, “ 10	2–18	12–14 (75–90)	10 m. (50–60); 16 m. (*ca.* 80)
	10, “ 17	1–12	9–10 (70–85)	8 m. (65–76); 11 m. (50–60)
	12, “ 19	2–14	*ca.* 8 (85–90)	6 m. (65–75); 10 m. (40–50)
	14, “ 20	2–14	6–8 (70–80)	4 m. (*ca.* 10); 10 m. (40–50)
	21, “ 24	1–9	5–6 (80–90)	4 m. (60–70); 7 m. (70–80)
20°	5, June 11	1–12	5–6 (70–90)	4 m. (40–50); 7 m. (60–70)
	7, “ 12	1–10	8–9 (90–95)	7 m. (60–70); 10 m. (*ca.* 90)
	11, “ 17	1–11	6–7 (*ca.* 95)	5 m. (80–90); 8 m. (80–90)
	13, “ 19	1–6	3–4 (*ca.* 95)	2 m. (30–35); 5 m. (70–80)
	18, “ 23	2–12	*ca.* 4 (*ca.* 50)	2 m. (20–25); 6 m. (25–35)
	21, “ 24	1–8	3–4 (90–95)	2 m. (15–20); 5 m. (80–90)
22°	5, June 11	1–12	3–4 (*ca.* 90)	2 m. (2–3); 5 m. (20–25)
	7, “ 12	1–8	6–7 (*ca.* 95)	5 m. (70–80); 8 m. (*ca.* 90)
	11, “ 17	1–10	*ca.* 4 (*ca.* 95)	3 m. (80–90); 5 m. (*ca.* 90)
	13, “ 19	1–5	2–3 (80–90)	1 m. (1–2); 4 m. (5–10)
	16, “ 22	½–4	3–3½ (65–75)	2½ m. (60–70); 4 m. (50–60)
	21, “ 24	½–3½	2–3 (75–90)	1½ m. (35–40); 3½ m. (60–70)

Temperature.	Number of Lot and Date.	Range of Exposures.	Optimum Exposures with Results.	Exposures Next Above and Next Below Optimum with Results (% Blastulæ).
		Min.	*Min. % blastulæ*	
24°	5, June 11	1–12	2 m. (60–65)	1 m. (0); 3 m. (15–20)
	7, " 12	1–6	3–4 (90–95)	2 m. (30–35); 5 m. (40–50)
	16, " 22	½–3½	2–2½ (80–85)	1½ m. (*ca.* 50); 3 m. (10–20)
	17, " 22	½–3	1½–2 (80–95)	1 m. (25–35); 2½ m. (40–50)
	21, " 24	½–3	1½–2 (*ca.* 90)	1 m. (5–10); 2½ m. (50–60)
26°	8, June 13	1–5	*ca.* 2 (few: 5–10)	1 m. (*ca.* 5); 3 m. (0)
	17, " 22	½–3	1–1½ (70–80)	½ m. (<1); 2 m. (10–15)
	18, " 23	½–3	1 (20–30)	½ m. (1–2); 1½ m. (5–10)
	19, " 23	½–2½	1 (30–40)	½ m. (<1); 1½ m. (0)
	20, " 24	½–2	1 (80–90)	½ m. (5–10); 1½ m. (30–35)
28°	8, June 13	1–4	1 (*ca.* 5)	2 m. (0)
	18, " 23	½–2	½ (10–15)	1 m. (0)
	19, " 23	½–2	½ (25–35)	1 m. (0)
	20, " 24	½–2	½ (80–90)	1 m. (<1)

THE BEHAVIOR OF SOME SOIL INSECTS IN GRADIENTS OF EVAPORATING POWER OF AIR, CARBON DIOXIDE AND AMMONIA.[1]

CLYDE C. HAMILTON.

PAGE.
I. Introduction ... 159
II. Methods of Study and Material ... 159
  1. Methods of Study ... 159
  2. Material ... 164
III. Experimental Results ... 165
  1. Larvæ ... 165
    A. Controls ... 165
    B. Reactions in Evaporation and Temperature Gradients ... 166
    C. Reactions in Carbon-Dioxide Gradients ... 172
  2. Adults ... 173
    A. Controls ... 173
    B. Reactions in Evaporation and Temperature Gradients ... 173
    C. Reactions in Ammonia Gradients ... 176
    D. Reactions in Carbon-Dioxide Gradients ... 177
IV. Summary and Discussion of Conclusions ... 177
V. Acknowledgments and Bibliography ... 181

I. INTRODUCTION.

The study of experimental ecology has started only within comparatively recent years and very little work has been done upon the relation of evaporation to the life economy of animals. The importance of the rate of evaporation as an environmental factor has been discussed by Shelford ('14*a*, *b*, *c*). Since evaporation is determined by the rate of air movement, humidity, temperature, pressure, and indirectly by illumination, it serves as an index of the general weather conditions. By varying any one of the factors controlling it, the rate of evaporation will be changed. The purpose of this paper is to show the behavior of some soil insects in evaporation, carbon dioxide, and ammonia gradients under experimental conditions.

II. METHOD OF STUDY AND MATERIAL.

1. *Method of Study.*

The method and equipment used in establishing the evaporation gradients was essentially the same as that described by

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[1] Contribution from the Zoölogical Laboratory of the University of Illinois, No. 83.

Shelford and Deere ('13), except for the method of measuring and controlling the rate of air flow. This is new and will be described in some detail. The supply of air was secured from the University high pressure supply and was reduced from about 80 pounds to 5 pounds by a Mason reducing valve, which gave a constant pressure irrespective of the amount of air used. At room temperature, about 70° F., the air had a relative humidity of 18 to 20 per cent. The moisture was removed from the air by passing it through sulfuric acid filters, then through a glass-wool filter to remove any trace of the acid, a coiled aluminum pipe to regulate the temperature, and finally to the apparatus for controlling the rate of air flow. The sulfuric acid filters consisted of six chloride jars, 18 by 3 inches, filled with moderately fine pumice stone saturated with sulfuric acid. The medium moist air and saturated air was passed through glass-wool filters and then moistened by blowing it over or bubbling it through water in wash bottles. By passing the air into the bottles through glass tubes and regulating the height of the opening above the water almost any per cent. of saturation could be obtained. From the wash bottles the air passed through coiled aluminum tubes for regulating the temperature and then to the apparatus for regulating the rate of air flow.

The experimental cage for observing the behavior and modification of the insects, and the glass tubes for testing their resistance to evaporation and gases, were the same as those described by Shelford and Deere ('13). The experimental cage was 30 cm. long, 6.5 cm. wide, and 2.5 cm. deep. The glass tubes were each 21 cm. long and 3.2 cm. inside diameter. The lower third of each tube was filled with paraffin upon which sand had been sifted while it was still warm. From each of the tubes the air passed to a chamber containing a Livingston porous cup atmometer so that the evaporation could be recorded while the animals were being observed. For testing the relative humidity of the air two long chemical thermometers, graduated to 0.5 of a degree, were inserted with the bulb end inside a glass tube about 20 centimeters long and 2 centimeters in diameter, through which the air was allowed to flow. The wet bulb thermometer was provided with a wick which connected with a vial of distilled water.

Glass Y's were inserted in each air line between the aluminum coils and the apparatus for regulating the air flow. By means of these Y's, cross connections of the different air lines could be made or carbon dioxide could be introduced into the air. The carbon dioxide was obtained from a tank of compressed carbonic acid gas. The pressure was reduced and regulated by the use of a pressure reducing valve. The gas passed from the pressure gage through a rubber tubing to a two-way valve. By regulating the size of these openings different proportions of the gas could be passed into any two of the three air lines. The percentage of carbon dioxide in the air was determined by collecting the mixture in a burette over water and absorbing the carbon dioxide with a solution of sodium or potassium hydroxide in a Hemple pipette. Only a little work was done with ammonia and no method was used for determining the per cent. in the air. Different gradients of ammonia air were secured by bubbling the air in each of two lines through water bottles containing 1,000 c.c. of water to which had been added 30 c.c. and 60 c.c. respectively of a $1/10N$ solution of ammonia. Air from each of these lines turned moist litmus paper in a few seconds.

The apparatus for measuring and regulating the air flow, in the different thirds of the cage, is new and was designed by Professor V. E. Shelford and the writer. The principle involved is that of measuring, by means of an inclined manometer, the static pressure produced in a box or cylinder when the outlet is smaller than the inlet. The apparatus, Fig. 1, consists of three similar parts, one part for each third of the cage. The air enters the cylinders through the back, first, however, passing up to the top of the back-board where there is a screw-clamp for regulating the flow. Fig. 2 is a diagrammatic longitudinal section of the cylinder showing its connections with the inclined manometer. The cylinder is divided into two halves, each 3 inches long and 3 inches in diameter, which are constructed with a rim around the outside of the adjacent ends in such a way that they can be fastened together with thumbscrews. A thin circular diaphragm or disk, with a small hole in the center, is placed between the two adjacent ends and the connections are made air tight by a rubber gasket on each side. The opposite ends of the cylinder have an inlet opening (*A*) and an outlet opening (*B*), each three eighths of an inch in diameter. On the upper side of each half of the cylinder are two openings (*E*) and (*F*) which are one fourth of an inch in diameter. These openings connect by rubber tubing to ends (*X*) and (*Y*) respectively of the inclined manometer.

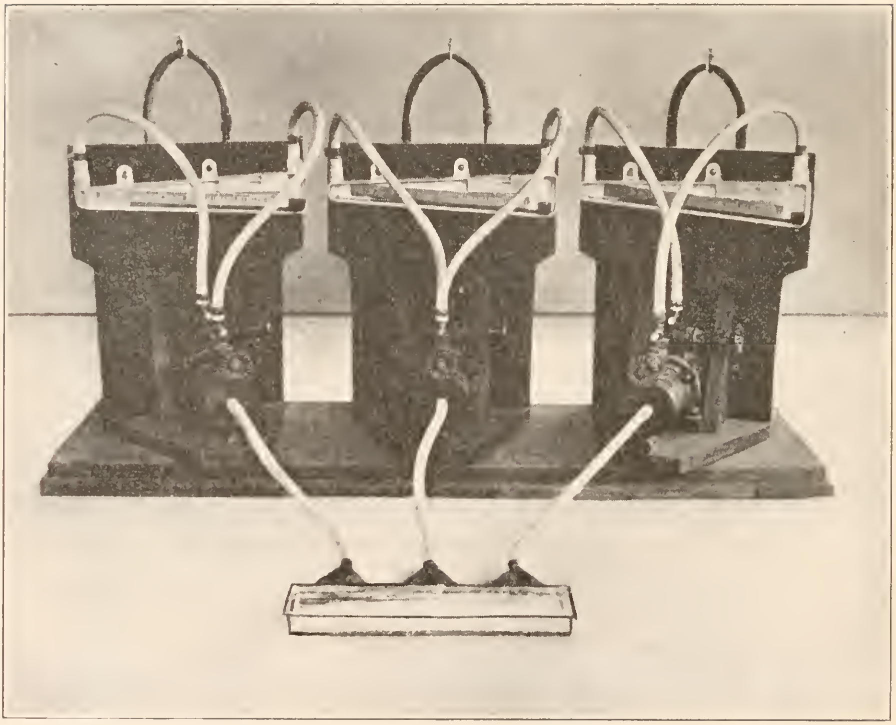

FIG. 1. Showing the three gages in position and connected with the gradient cage. The metal hood with symmetrical electric lights is removed. The valves make it possible to measure the pressure on either side of the diaphragm separately and thus check the results when both sides are connected.

The liquid used in the manometer was a red oil but the scale was corrected to read in millimeters of water. The amount of air entering chamber (1) through opening (*A*) is regulated by a screw-clamp (*T*) on the rubber tubing (*S*). The opening (*C*) in the diaphragm (*D*) should be in the center and may be any size smaller than openings (*A*) and (*B*). Since opening (*C*) is smaller than opening (*A*) the air cannot escape from the chamber as readily as it enters and as a result static pressure is produced. This pressure is indicated through opening (*E*) on the inclined manometer. Further, since opening (*C*) is smaller than opening (*B*) the air escapes through (*C*) into chamber (2) the same as if into the free atmosphere or without exerting any static pressure. If, however, the rubber tubing connecting opening (*B*) with the experimental cage is long or crooked, static pressure may be exerted in chamber (2) due to the friction of the air in the tubing, or the tubing may become kinked or closed in which case the air would pass out through opening (*E*) and the inclined manometer. To remedy this possible chance of error it is advisable to have chamber (2) connected with end (*Y*) of the manometer. If this is done only the difference in static pressure between chambers (1) and (2) is indicated or the actual amount of pressure exerted by the air flowing through opening (*C*). By using diaphragms with different-sized openings different volumes of air can be

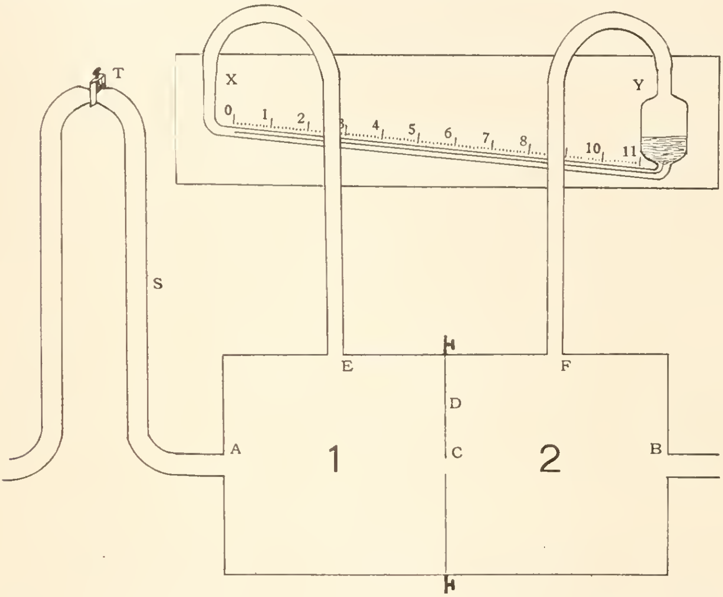

FIG. 2. Showing a diagrammatic section of the diaphragm chambers and gage. For description see text page 161. The number of units in the scale is here reduced to 11 for convenience. The number in the gages in use is 17, each representing one millimeter of water.

measured. Openings almost as large as the inlet opening may be used for large flows of air and smaller openings for smaller flows of air.

The amount of air in pounds per second flowing through (*C*) may be determined by the following formula (Durley, '06); $.6299\, CD^2 \sqrt{I/T}$, in which *C* is a constant approximately .602 for small openings and slight pressures, *D* is the diameter of the opening in the diaphragm in inches, *I* is the inches of water displaced, and *T* is the absolute temperature *F.* (absolute 0 is − 459° F.). The constant *C* varies slightly for different-sized openings and different pressures, but this variation is so small, however, that it may be considered as negligible in computing the volume of air passing through each third of the experimental cage. The diameter of the opening in the diaphragms, for all of the experiments performed, was five sixteenths of an inch. The static pressure was recorded in millimeters instead of inches of water. By changing the millimeters to inches, substituting the necessary data in the formula and solving, the volume of air in pounds per second may be obtained. It was thought desirable, however, to give the volume in liters of air per minute. This may be obtained by multiplying the pounds per second by 60 to get the pounds per minute, then by 454 to get the grams per minute, and dividing by 1.1265, which is approximately the number of grams in one liter of air at 70° F. and a barometric pressure of 29 inches.

2. *Material.*

The material studied consisted of full grown larvæ and adults of the family Carabidæ. The following species of larvæ were used in the experiments: *Evarthrus sodalis* Lec., *Harpalus vagans* Lec., *Harpalus erythropus* Dej., *Pterostichus corvinus* Dej., and *Amara avida* Say, The species of adults studied were *Evarthrus sodalis* Lec., *Harpalus erythropus* Dej., *Harpalus pennsylvanicus* Dej., *Pterostichus corvinus* Dej., *Pterostichus stygicus* Say, *Amara avida* Say, *Anisodactylus nigrita* Dej., *Patrobus longicornis* Say, and *Patrobus placidus* Say. The material was collected at various times throughout the year and was kept in two-ounce tin boxes partly filled with moist soil. A few of the larvæ were subjected to special treatment, which will be discussed under the experiments. The majority of the experiments on the larvæ were performed with the species *Evarthrus sodalis*. The larvæ of this species are about an inch long, vigorous, active, easily collected, and could be found in a variety of situations. They were dug in all kinds of situations, from dry or wet, black soil in corn fields to wet, sandy or humus soil close to creek beds. The larvæ of the genus *Harpalus* were mostly collected in wet, sandy soil close to a creek bed; but they were also taken in drier and heavier soil. The larvæ of the genus *Amara* were dug from wet, sandy soil close to a creek and from moist, black soil in the bottom of a depression which contained considerable humus. Larvæ of the genus *Pterostichus* were dug from situations similar to those of *Evarthrus sodalis*. The adults were collected at various places.

III. Experimental Results.

About one hundred and twenty-five experiments were performed at various times during the fall, winter, and spring of 1915–16. Only a few representative ones, however, will be given and discussed, together with any peculiar variations which may need to be noted.

1. *Larvæ.*

A. Controls.—The controls, which were conducted in still air, were principally of two types as far as results were concerned. The larvæ were either active, crawling from one end of the cage to the other, but occasionally stopping at either end to try to crawl out, or they soon became quiet and remained in any part of it and appeared to rest or sleep. At temperatures of about 18° to 22° C. the larvæ were usually active and crawled from end to end of the experimental cage. At temperatures below 18° C. they were often inactive and remained quiet unless conditions were otherwise unfavorable. At temperatures of 23° C. and above the larvæ were sometimes inactive but not at ease. The increased temperature seemed to have a depressing effect and the larvæ acted tired and uneasy. Since in the normal controls the larvæ were either quite symmetrical in their movements or were quiet, it was not thought necessary to give the control for each experiment. Graph 1 shows the control for an *Evarthrus sodalis* larva at a temperature of 20.5° C. The distance from the right to left represents the length of the cage and the vertical distance time. Graph 8 shows the control of another *Evarthrus sodalis* larva with a temperature of 23° C. at the start and 26.5° C. at the end.

B. Reaction in Evaporation and Temperature Gradients.—The experiments on evaporation were varied by changing the relative humidity, the temperature, and the rate of air flow. The flow of air used, unless otherwise stated, was 13.3 liters per minute. This volume of air was secured by using a diaphragm with an opening five sixteenths of an inch in diameter and a displacement of three millimeters of water.

Graph 2 shows the reaction of two *Evarthrus sodalis* larvæ to dry air at a temperature of 21° C. The avoidance of the dry air was very sharp and decided by one of them. The other larva rushed into the dry air, was soon overcome and was unable to get out. It squirmed and rolled about, became less active and was dead at the end of forty minutes. It had shrunken considerably, due to the withdrawal of water, and had turned a dirty yellow color. The larva which avoided the dry air was apparently as active at the end of the experiment as at the beginning. At lower temperatures the larvæ were generally not so active and did not avoid the dry air so sharply. Graph 3 shows the reaction of an *Evarthrus sodalis* larva to dry air at a temperature of 17° C. In the wet air it acted normal, except for some sluggishness. In the medium and dry air it squirmed and rolled about but was not affected as much as when the temperature was higher. Graph 4 shows the reaction of an *Evarthrus sodalis* larva, which had been outside in freezing weather for five days, to a high temperature and a low percentage of moisture. The behavior was different from those in dry air at lower temperatures in that the larva was sluggish, depressed and appeared fatigued. The effect appeared to be very similar to that of carbon dioxide, which will be described later. The graph shows the movement of the larva for forty minutes, the second twenty minutes starting at (X). At the end of the forty minutes the larva was considerably shrunken, was a creamy yellow color in the membranous areas, and appeared to be dead. It began to revive in a little over an hour, but never fully recovered. Graph 5 shows the reaction of an *Evarthrus sodalis* larva, which had been outside in freezing and thawing weather for eighteen days, to dry air and a temperature of 22.5° C. There was a tendency in this larva to avoid the driest air. This is shown not so much by the time spent in the dry air but by the fact that in only one instance did the larva reach the end of the cage containing the dry air. It was in the dry air for a greater length of time than in the other air but this was due to its squirming, twisting and slower movements. Graph 6 shows the reaction of an *Evarthrus sodalis* larva, which had been outside in frozen ground for five days, to dry air at a temperature of 16.5° C. The larva was brought in from the outside and allowed to revive for a few minutes before the experiment was started. The larva behaved more normal than those in graphs 4 and 5 and showed a greater tendency to select air suitable to itself.

Graph 7 shows the reaction of an *Evarthrus sodalis* larva to different rates of air flow at a temperature of 18° C. and a relative humidity of about 44 per cent. Although the larva was affected by the different gradients of evaporation thus produced, it showed no tendency to select its air. The flow of air in the different thirds of the cage was 0, 13.3, and 69.5 liters, respectively, per minute. Graph 8 shows the control of an *Evarthrus sodalis* larva at a temperature of 23° C. at the start and 26.5° C. at the end. Graphs 9, 10, and 11 show the reactions of three *Evarthrus sodalis* larvæ to air of practically the same temperature and relative humidity in corresponding parts of the cage but with different rates of air flow for each experiment. In graph 9, with an air flow of 13.3 liters per minute or 3 millimeters of water displaced, the larva was affected by the dry air but showed little tendency to avoid it. In graph 10, with an air flow of 19.0 liters per minute or 6 millimeters of water displaced, the larva showed no tendency to avoid the dry air. It was, however, affected, and became sluggish and less active as the experiment continued. Graph 11 shows the reaction of an *Evarthrus sodalis* larva to an air flow of 22.4 liters per minute or a displacement of 9 millimeters of water. With this rate of air flow the larva selected the wet air and was in much better con-

Chart I.

Graph 1 shows the control of an *Evarthrus sodalis* larva in still air at a temperature of 20.5° C.[1]

[1] Where different temperatures, relative humidities, rates of air flow, and percentages of gas are given, the one on the left side of the cage is named first, the middle second, and that on the right side last.

Graph 2 shows the reaction of two *Evarthrus sodalis* larvæ to dry air at a temperature of 21° C. Relative humidity of the air, wet 81 per cent., medium 47 per cent., dry 17 per cent. Rate of air flow 13.3 liters per minute. The larvæ had been kept inside at a temperature of 20 to 21 degrees C.

Graph 3 shows the reaction of an *Evarthrus sodalis* larva to dry air at a temperature of 17° C. Relative humidity of the air, wet 82 per cent., medium 55 per cent., dry 4 per cent. Rate of air flow 13.3 liters per minute. The larva had been kept inside at a temperature of 20° to 21° C.

Graph 4 shows the reaction of an *Evarthrus sodalis* larva, which had been kept outside in freezing weather for five days, to a high temperature and a low percentage of moisture. The experiment ran for forty minutes, the second half of the graph starting at "X." Temperature of the air, wet 28° C., medium 26.5° C., dry 25.5° C. Relative humidity of the air, wet 40 per cent., medium 26 per cent., dry 1 per cent. Rate of air flow 13.3 liters per minute.

Graph 5 shows the reaction of an *Evarthrus sodalis* larva, which had been kept outside in freezing and thawing weather for 18 days, to dry air and a temperature of 22.5° C. Relative humidity of air, wet 95 per cent., medium 63 per cent., dry 4 per cent. Rate of air flow 13.3 liters per minute.

Graph 6 shows the reaction of an *Evarthrus sodalis* larva, which had been outside in freezing weather for five days, to dry air and a temperature of 16.5° C. Relative humidity of the air, wet 94 per cent., medium 52 per cent., dry 4 per cent. Rate of air flow 13.3 liters per minute.

Graph 7 shows the reaction of an *Evarthrus sodalis* larva to different rates of air flow. Temperature 18° C. Relative humidity of air, 44 per cent. at the start and 39 per cent. at the end. Rates of air flow, 0, 13.3, and 69.5 liters per minute. Evaporation from atmometers for 20 minutes, 0, .94 c.c. and 2.25 c.c.

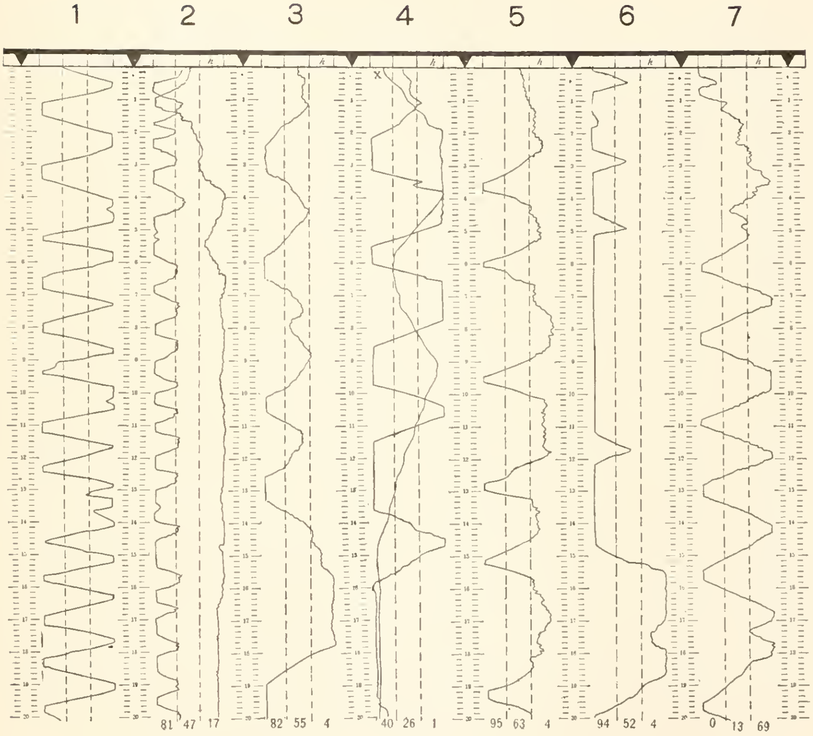
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CHART II.

Graph 8 shows the control of an *Evarthrus sodalis* larva in still air at a temperature of 23° C. at the start and 26.5° C. at the end.

Graph 9 shows the reaction of an *Evarthrus sodalis* larva to an air flow of 13.3 liters per minute at a temperature of 22.5° C. Relative humidity of air, wet 54 per cent., medium 41 per cent., dry 7 per cent. Evaporation for 20 minutes, wet air .50 c.c., medium air .69 c.c., dry air 1.42 c.c.

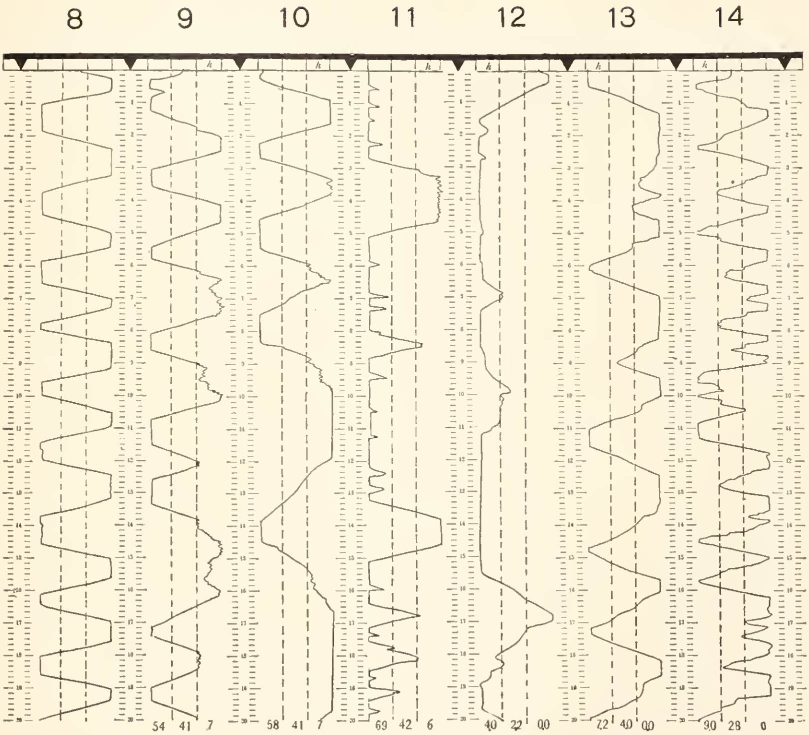
Graph 10 shows the reaction of an *Evarthrus sodalis* larva to an air flow of 19.0 liters per minute and a temperature of 22° C. Relative humidity of air, wet 58 per cent., medium 41 per cent., dry 7 per cent. Evaporation for 20 minutes, wet air .60 c.c., medium air 1.00 c.c., dry air 1.60 c.c.

Graph 11 shows the reaction of an *Evarthrus sodalis* larva to an air flow of 22.4 liters per minute and a temperature of 21.5° C. Relative humidity of air, wet 69 per cent., medium 42 per cent., dry 6 per cent. Evaporation for 20 minutes, wet air .54 c.c., medium air 1.07 c.c., dry air 1.75 c.c.

Graph 12 shows the reaction of an *Evarthrus sodalis* larva to a low percentage of carbon dioxide. Temperature 18° C. Relative humidity of air, 75 per cent. Rate of air flow 13.3 liters per minute. Per cent. of carbon dioxide in the different thirds of the cage, 4.0 (at left), 2.2, and 0.0.

Graph 13 shows the reaction of an *Evarthrus sodalis* larva to an increase in the per cent. of carbon dioxide. Temperature 18° C. Relative humidity of the air, 75 per cent. Rate of air flow 13.3 liters per minute. Per cent. of carbon dioxide in the different thirds of the cage, 7.2 (at left), 4.0, and 0.0.

Graph 14 shows the reaction of an unknown species of larva to a still higher percentage of carbon dioxide. Temperature 19° C. Relative humidity of the air, 95 per cent. Rate of air flow 13.3 liters per minute. Per cent. of carbon dioxide in the different thirds of the cage, 9.0 (at left), 2.8, and 0.0.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dition at the end of the experiment than either of the larvæ used in graphs 9 and 10.

In testing the resistance of larvæ to evaporation, in the glass tubes previously mentioned, one larva died at the end of 115 minutes' exposure in the tube containing the dry air at a temperature of 22.5° C. and a relative humidity of 9 per cent. The larvæ in the tubes containing the medium air, relative humidity 64 per cent. and temperature 22.5° C., and the wet air, relative humidity 95 per cent. and temperature 22.5° C., were not affected at the end of two hours. At a temperature of 23° to 24° C. another larva lived 75 minutes in the tube containing air with a relative humidity of 4 per cent., another one 120 minutes in the tube containing medium air with a relative humidity of 40 per cent., while in the tube containing the wet air, relative humidity about 80 per cent., the larva was slightly shrunken and inactive at the end of 160 minutes but not dead. The temperature had risen to about 26° C. when the experiment was discontinued. In testing the resistance of larvæ to gradients of evaporation, produced by different rates of air flow, at a temperature of 21.5° C. and a relative humidity of 7 per cent., one larva died after 35 minutes' exposure with an air flow of 16.4 liters per minute or 4.5 millimeters of water displaced. During this time 2.1 c.c. of water had evaporated from the porous cup atmometer. In air of the same temperature and relative humidity but an air flow of 13.3 liters per minute or 3 millimeters of water displaced, another larva died at the end of 68 minutes, with an evaporation of 3.2 c.c. of water from the porous cup atmometer. With an air flow of 9.3 liters per minute or 1.5 millimeters of water displaced a third larva died after 90 minutes' exposure with an evaporation for this period of 3.4 c.c. of water from the atmometer cup. The air was the same temperature and relative humidity as in the previous two experiments. The larva in the tube containing the larger flow of air was considerably disturbed from the first and rolled and twisted about. The larvæ in the other two tubes did not behave so violently but gradually became sluggish and less active until they died.

C. Reactions in Carbon Dioxide Gradients.—Graphs 12, 13 and 14 show the reaction of larvæ to air containing different percentages of carbon dioxide at a temperature of 18° to 19° C. and a relative humidity of 75 to 95 per cent. In graph 12, with carbon dioxide percentages of 4.0, 2.2, and 0.0 respectively, an *Evarthrus sodalis* larva selected the air containing the largest amount of carbon dioxide. The larva used in graph 13, with carbon dioxide percentages of 7.2, 4.0, and 0.0 respectively, showed no marked preference for either the carbon-dioxide air or the carbon-dioxide-free air. In graph 14 an unknown species of larva showed a decided negative reaction to air containing 9.0 per cent. of carbon dioxide. No experiments were performed to determine the time of death of the larvæ in different amounts of carbon dioxide. In general, however, for mixtures of the gas up to 11 or 12 per cent. the larvæ were not violently affected. In air containing from about 7 to 12 per cent. of carbon dioxide they were sluggish and inactive and appeared fatigued. In percentages below this they sometimes selected the air containing the carbon dioxide and sometimes avoided it. They were not, however, much affected, if any, by low percentages of the gas. There was no violent squirming and twisting produced by the carbon dioxide but it seemed to have a depressing effect. The larvæ were sluggish, inactive, and appeared exhausted.

2. *Adults.*

A. Controls.—The behavior of the adults, both in the experiments and in the controls, was more irregular than that of the larvæ. They moved quicker and did not have the blind tendency to continue in the direction they were going if the conditions became unfavorable. Graph 15 shows two ten-minute controls for adults. The first ten minutes is the control for an *Evarthrus sodalis* adult and the last ten minutes the control for a *Patrobus longicornis* adult. Temperature 21° C. and no air flow. The *Evarthrus* adults showed a uniform tendency to run from one end of the cage to the other, while the majority of the other species often ran hesitatingly about for a time and then settled down and remained quiet, usually at one end of the cage.

B. Reactions in Evaporation and Temperature Gradients.—A number of experiments were performed with different species, but only a few can be given here. Graph 16 shows the reaction

CHART III.

Graph 15 shows the control for two species of adult Carabids. The first one half is the control for an *Evarthrus sodalis* adult and the second one half for a *Patrobus longicornis* adult. Temperature 21° C. No air flow.

Graph 16 shows the reaction of a *Pterostichus stygicus* adult to dry air and a temperature of 20.5° C. Relative humidity of air, wet 95 per cent., medium 86 per cent., dry 13 per cent. Rate of air flow 13.3 liters per minute.

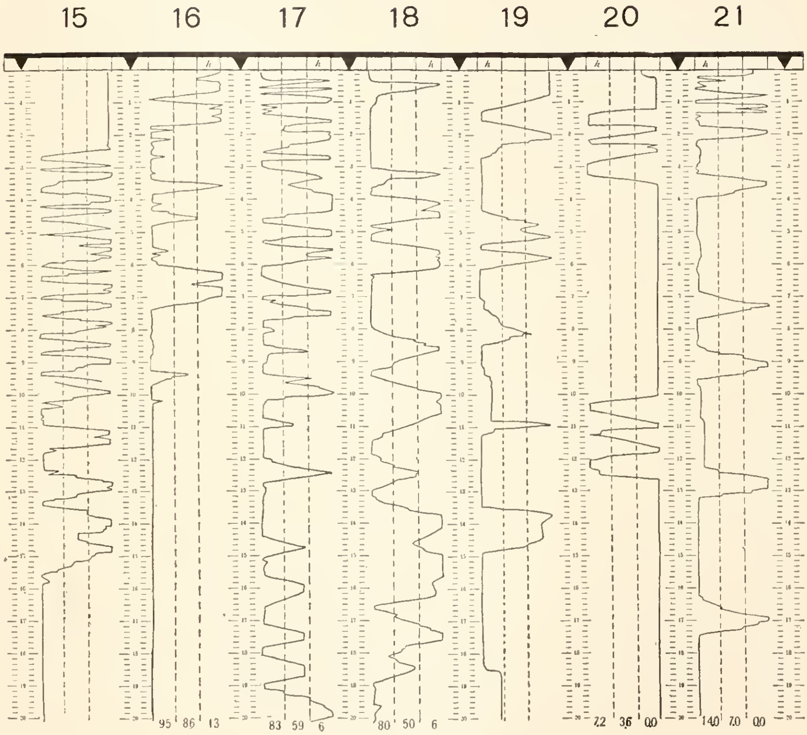
Graph 17 shows the reaction of a *Pterostichus corvinus* adult to dry air at a temperature of 19.5° C. Relative humidity of air, wet 83 per cent., medium 59 per cent., dry 6 per cent. Rate of air flow 13.3 liters per minute.

Graph 18 shows the reaction of a *Pterostichus corvinus* adult to dry air and a temperature of 16.5° C., with an increase in the rate of air flow to 19.0 liters per minute. Relative humidity of air, wet 80 per cent., medium 50 per cent., dry 6 per cent.

Graph 19 shows the reaction of an *Evarthrus sodalis* adult to gradients of ammonia. Temperature 22° C. Relative humidity of air, 98 per cent. Rate of air flow 13.3 liters per minute.

Graph 20 shows the reaction of a *Pterostichus stygicus* adult to gradients of carbon dioxide. Temperature 22° C. Relative humidity of the air, 90 per cent. Rate of air flow 13.3 liters per minute. Percentage of carbon dioxide in the different thirds of the cage, 7.2, 3.6, and 0.0.

Graph 21 shows the reaction of an *Evarthrus sodalis* adult to an increase in the percentage of carbon dioxide. Temperature 22.5° C. Relative humidity of the air, 86 per cent. Rate of air flow 13.3 liters per minute. Percentage of carbon dioxide in the different thirds of the cage, 14.0, 7.0, and 0.0.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of a *Pterostichus stygicus* adult to dry air at a temperature of 20.5° C. and an air flow of 13.3 liters per minute. The adult showed a tendency to move about at first but soon became inactive and remained quiet during the remainder of the experiment. It was active and in good condition when the experiment was discontinued. Graph 17 shows the reaction of a *Pterostichus corvinus* adult to dry air at a temperature of 19° C. and an air flow of 13.3 liters per minute. The adult did not show as decided a reaction against the dry air as the adult in the previous experiment but at the end of about eight minutes a preference was worked out for the wet air. Graph 18 shows the reaction of an adult of the same species to dry air at a still lower temperature, 16.5° C., and an increase in the rate of air flow to 19.0 liters per minute. Although the adult was restless and uneasy during the experiment, it showed little tendency to select the wet air.

To test the resistance of the adults to evaporation, three adults were put in the glass tubes with air flowing through them at a temperature of 21° C. at the beginning and 23° to 25° C. at the end, and a relative humidity at the end of 30 per cent. In the tube with an air flow of 13.3 liters per minute the adult died after 28 hours' exposure, during which time 70 c.c. of water had evaporated from the porous cup atmometer. In the tube with an air flow of 19.0 liters per minute the adult was dead at the end of 30 hours, during which time 77 c.c. of water had evaporated from the atmometer cup. In the third tube, with an air flow of 22.4 liters per minute the adult was dead at the end of 24 hours, during which time 82 c.c. of water had evaporated from the atmometer cup.

C. Reaction in Ammonia Gradients.—A few experiments were performed with air containing ammonia to determine the reaction of the adults to this gas. To obtain the gradients of ammonia in the air it was bubbled through water containing different amounts of the gas in solution. No quantitative determinations of the amount of ammonia in the air were made. Graph 19 shows the reaction of an *Evarthrus sodalis* adult to gradients of ammonia at a temperature of 22° C. and a relative humidity of 98 per cent. In this instance the avoiding reaction against the ammonia was rather decided. The majority of the individuals tested showed considerable signs of disturbance and discomfort but they were not very strongly negative to ammonia; one or two continued to remain in the ammonia air although they were not at ease. The adults moved hesitatingly and at random in the ammonia air, stroking the antennæ and occasionally the abdomen.

D. Reaction to Carbon Dioxide.—The reaction of the adults to gradients of carbon dioxide was not very different from that of the larvæ. Graph 20 shows the reaction of an adult *Pterostichus stygicus* to air containing carbon dioxide gradients of 7.2, 3.6, and 0.0 per cent. with a temperature of 22° C. and a relative humidity of 90 per cent. The adult tried the carbon-dioxide-free air several times, but each time came to rest in the air containing the largest amount of the gas. Graph 21 shows the reaction of an adult of the same species to air containing carbon dioxide gradients of 14, 7, and 0.0 per cent. with a temperature of 22.5° C. and a relative humidity of 86 per cent. In this instance the adult showed a distinct negative reaction to the flow of air containing the largest per cent. of carbon dioxide, spending most of its time in the carbon-dioxide-free air. In most cases where the carbon dioxide was about 6 or 7 per cent., or higher, the adults either avoided it or were uneasy and distressed. With lower percentages of the gas very little signs of uneasiness were shown.

IV. Summary and Discussion of Conclusions.

A thorough understanding of the behavior of the soil insects to evaporation, temperature, and gases found in the soil necessitates a knowledge of the conditions existing in their habitat. This includes the physical condition of the soil, the temperature, the moisture, and the gases. The physical structure of the soil, *i. e.*, size of soil particles, porosity, amount of organic material, etc., is important, since it is upon these that the soil moisture, temperature, and aeration is dependent. The temperature of the soil is fairly constant. It does not have the range of variation and is not subjected to the sudden changes encountered in the atmosphere. This is more noticeably so in soil which is moderately moist to wet and is due, to a large extent, to the great amount of heat used in the evaporation of water. Thus considerable heat can be used in the evaporation of moisture from wet or moist soil and the temperature of the soil itself will remain almost unchanged. On the other hand, in dry compact soil most of the heat is absorbed and the temperature of the soil is raised accordingly.

The moisture in the soil is derived largely from precipitation, but part of it may come from the adjacent soils or rock. The amount which a soil will retain is dependent upon the porosity, the physical structure, the litter covering the soil, and the growth of vegetation. A soil with a living vegetative cover loses moisture, both through direct evaporation and absorption by its vegetation, much faster than bare moist soil. The organic debris on the surface also conserves the moisture by the formation of a blanket or mulch. Evaporation is further retarded by the use of windbreaks or hindering the movement of the air over the soil, as has been shown by numerous experiments with windbreaks. Adams ('15) in discussing the soil moisture says: "McNutt and Fuller ('12) have made a study of soil moisture at 3 inches (7.5 cm.) and at 10 inches (25 cm.) below the surface in an oak-hickory forest, at Palos Park, Illinois. They found that the percentage of water to the dry weight of the soil at the 3-inch level averaged 18.9 per cent. and at 10 inches was 12.5 per cent. of the dry weight of the soil. The greater moisture near the surface is due to the humus present in this layer."

The ventilation and amount of air in the soil is dependent upon its physical properties, temperature and moisture. Dry soil, according to Hilgard ('06) contains from 35 to 50 per cent. its volume of air, and in moist or wet soil this space is replaced by water. Thus the conditions influencing the amount of water present have a very important influence upon aeration. The rapidity with which ventilation occurs is dependent upon the porosity and temperature; the greater the porosity and the higher the temperature, the more rapid the change. The amount of carbon dioxide in the soil is partly dependent upon the ventilation, the moisture, and the amount of decaying organic material. Since carbon dioxide is very soluble in water, it is found in the soil moisture in much larger proportions than in the atmosphere.

This concentration for different kinds of soil is shown by the following table by Bassungault and Lewy (Van Hise, '04).

Character of Soil Air.	CO_2 in 10,000 Parts by Weight.
1. Sandy subsoil of forest	38
2. Loamy subsoil of forest	124
3. Surface soil of forest	130
4. Surface soil of vineyard	146
5. Pasture soil	270
6. Rich in humus	543

From this we see that the amount may reach as high as 5 or 6 per cent. in some situations.

Little is known concerning the occurence of ammonia in soil air. It usually occurs in very small quantities but may occasionally be noticeable in places where there is much decaying organic matter. The small amounts of ammonia which may occur in the soil are usually held in electrolytic combination with the soil itself and do not occur free in the air.

The above is a very brief survey of some of the physical conditions which must be encountered by soil inhabiting insects. If we return to the experiments we will notice that the larvæ were very sensitive to evaporation, and especially so if the temperature was about 20° C. or above. This is what could be expected, since in their natural habitat the relative humidity of the air, in moist or wet soil, is not far below saturation and the temperature of the soil probably rarely goes above 20 to 23 degrees C., and then only in exposed, dry, hard soil in which one would not find the larvæ. It will also be noticed that of those larvæ which had been outside in freezing and thawing weather, the one experimented with at the lowest temperature behaved more normally and showed a greater tendency to select air probably suitable to its physiological condition. An increase in the rate of air flow, other conditions remaining the same, did not seem to affect the larvæ as much as an increase in temperature or a decrease in the relative humidity. They were probably affected more by the breathing in of the dry air and an increase in the temperature than by evaporation from the body surface. Their almost instantaneous detection of dry air and a high temperature would seem to point to this conclusion.

The increased temperature may have affected the larvæ by increasing their activity and metabolic processes, thus requiring a larger amount of air. If this air is dry, we can understand why the larvæ are more sensitive to dry air at the higher temperatures. On the other hand, if the temperature is lowered, their activity and metabolic processes are decreased, thus requiring a smaller amount of oxygen. In temperatures of 24° to 26° C. and above, the larvæ were often depressed, less active, and did not respond to the dry air so sharply. This may have been due to the fact that the temperature was increased above the point where their maximum activity occurred and that above this point an increased temperature had a depressing effect. In those experiments, in which the larvæ had been subjected to freezing weather for a number of days, the temperature at which the larvæ avoided the dry air the best was lowered from about 22° C. to 16° C. It was also noticed that larvæ collected early in the fall withstood high temperatures much better than those collected later.

The adults behaved somewhat similar to the larvæ in the experiments. They were more resistant to dry air and did not have the blind tendency to keep going ahead when the conditions became unfavorable. They often stopped and investigated the dry air, apparently sensing it with their antennæ. Their greater resistance to evaporation and temperature is natural, since they are usually found on the surface of the soil or near it, and are thus subjected to higher temperatures and drier air. Their increased chitinization of the body is also a greater protection from evaporation from the body surface.

The reaction of the larvæ and adults to carbon dioxide is not surprising when we consider their habitat and the amount of carbon dioxide found in the soil. No analyses of the moisture or the amount of carbon dioxide in the soil were made, but taking the figures available, the amount could not have been much below 4 per cent., which was selected by the larvæ in the experiments and which is probably their optimum. Insects are much more resistant to carbon dioxide than are warm-blooded animals and fishes (Shelford and Powers, '15) and can use practically all of the oxygen from a given quantity of air. This has been shown by Shafer ('11) and other workers. Grasshoppers were left in pure carbon dioxide for 15 hours and adults of *Passalus cornutus* for 24 hours and complete recovery resulted. Large percentages of the carbon dioxide caused violent twisting and movements of the larvæ for a few minutes and then they became inactive and sluggish or stupefied. No experiments to determine their resistance to carbon dioxide were performed.

The experiments with the gradients of ammonia showed that the adults were affected by the gas but that they did not always react advantageously to it. The percentages used were probably considerably larger than that ever encountered in nature. Under some conditions of decaying vegetable matter considerable ammonia may, however, be present in the air.

In general the experiments show that the reactions of the larvæ and adults to gradients of evaporation, temperature, and carbon dioxide are similar to what one would expect from a study of their habitat. Adams ('15) in speaking of soil conditions says: "The animals which thrive in the soil are likely to be those which tolerate a large amount of carbon dioxide and are able to use a relatively small amount of oxygen, at least for considerable intervals, as when the soil is wet during prolonged rains. . . . The optimum soil habitat is therefore determined, to a very important degree, by the proper ratio or balance between the amount of available oxygen and the amount of carbon dioxide which can be endured without injury."

IV. Acknowledgments and Bibliography.

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THE REACTIONS OF CERTAIN MOIST FOREST MAMMALS TO AIR CONDITIONS AND ITS BEARING ON PROBLEMS OF MAMMALIAN DISTRIBUTION.[1]

HOMER E. CHENOWETH.

PAGE.
I. Introduction 183
II. Habitat and Distribution 184
III. Experimental Study of the Relation of Environmental Factors to Distribution 185
  1. Method of Establishing Evaporation Gradients 186
  2. General Plan of Experiments 190
  3. Material 191
  4. Experimental Results 191
  5. General Discussion 197
IV. Summary 199
V. Acknowledgments and Bibliography 200

I. INTRODUCTION.

The reactions of animals to environmental factors in experiments indicate conditions suitable for them in nature. If such experiments are carried out carefully and at different periods of the life history, the reason for the presence of any animal in a particular environment may be explained in terms of measurable physical factors which it selects and avoids. It has been shown that insects, spiders and amphibians from moist and dry habitats respectively were sensitive to variations of the evaporating powers of the air. The physical factors that determine the rate of evaporation are humidity, pressure, wind and temperature, and these are measured in combination by instruments measuring evaporation. It is the main purpose of this paper to show that representatives of the mammalian group, wood mouse (*Peromyscus leucopsus noveboracensis* Fischer), react to variations in the evaporating power of air in a similar manner regardless of the cause of the variation. The local distribution of an animal is determined by its reactions to environmental factors and its geographic distribution is a function of the distribution of its local habitat, or of conditions indicated by its local habitat. In spite of the fact that most theories of distribution are based largely upon mammals we know of no studies of mammalian behavior bearing on this question. However if our knowledge of climate were complete, and if it were possible to bring representatives of all the land animals into the laboratory and test their reactions to physical factors at intervals through their life history a distribution map could probably be made fairly accurate for each species.

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[1] Contribution from the Zoölogical Laboratory, University of Illinois, No. 82.

II. Habitat and Distribution.

One of the commonest mammals of any woodland areas of Illinois is the white-footed wood mouse. The habitat of this subspecies is practically coextensive with the woodland. This mouse is sometimes found in the fields when the food supply is short in the forest or it may be tempted to a nearby cornfield. They may migrate to the forest edge or to open cleared places a mile or so from the woods during the summer. They seem to be able to adapt themselves to different moist environments and this is no doubt a great factor in determining their abundance though it seems that houses and barns are seldom if ever entered and it is certain that they never become a household pest. In the woods they are at home under any kind of rubbish and the roots of trees but they are not limited to the ground strata as they are sometimes found living in the trees. The range of the subspecies is from Novia Scotia to central Minnesota, thence south through the humid parts of eastern Nebraska and Kansas and eastward to the Atlantic.

The main purpose of these experiments was to determine the environmental factors that restrict the range of this mouse to the woodland. So many factors make up the environment of any animal and a change in one usually affects the others so that a study of this kind is a very difficult one.

Since the relative humidity is so much greater in the forest than on the prairie (Shimek '11) the writer shows in these experiments that the mouse is sensitive to this difference and reacts to any change in which the humidity is lower than that of the woodland in which its habitat is restricted. Since changes in the relative humidity directly affect the rate of evaporation which in turn affects the physiological processes the conclusion that the evaporating power of the air is the best index of environmental conditions of the white-footed woodland mouse as well as other land mammals seems evident.

This mouse is primarily a ground stratum animal and since relative humidity decreases from the ground upward one would expect the reactions of such a species to be more pronounced than those from the higher strata, and Shelford ('13) from a series of experiments found (1) animals react to air of a given rate of evaporation whether the rate of evaporation is due to moisture, temperature, or rate of movement; (2) the sign and degree of the reaction to the given rate of evaporation are in accord with the comparative rates of evaporation in the habitats from which the animals were collected though the reaction to evaporation due to temperature was usually sharper; (3) the animals of a given habitat are in general agreement in the matter and sign of the reaction; the minor differences which occur are related to vertical conditions and kind of integument; (4) there is a rough agreement between survival of time in an air of high evaporating power and kind of integument, but no agreement between survival of time and habitat when a number of species of the community are taken together.

Fuller ('11) and McNutt and Fuller ('12) made a comparative study of rates of evaporation in different kinds of forests in northern Illinois. Their figures show that the rate of evaporation was dependent upon the kind of forest and the following results were obtained. Taking 100 as a relative evaporation scale: Cotton wood dunes, 260 per cent.; oak dune, 127 per cent.; oak hickory, 115 per cent.; maple beach forest, 100 per cent. Shimek ('10, '11) made observations on the relative rate of evaporation on the prairies of western Iowa, and his results show that the rate of evaporation is much greater in exposed places than where there is shelter from the sun and wind. The mice apparently avoid higher rates of evaporation by staying in the forest.

III. Experimental Study of the Relation of Environmental Factors of Distribution.

Aron ('11), Rheinhard ('69) and Rubner ('90) have shown that evaporation directly affects the physiological processes of certain mammals and that if these processes were sufficiently interfered with death of the animal resulted.

1. *Method of Establishing Evaporation Gradients.*

The apparatus for this experiment was designed by Dr. V. E. Shelford and E. O. Deere, and for descriptions and drawings and method of establishing evaporation gradients see BIOL. BULL., No. 25, '13. Descriptions will be made only where changes were made in the apparatus.

The experimental cages for the gradient experiment were designed by Dr. V. E. Shelford. Since a much greater rate of air flow was used in these experiments the cages were made about three times as large as the designer used in his experiments. An extra division of wire mesh was inserted between the fish-tailed burner-shaped introducers and cage apartment. This served to make a more even distribution of the air over the cage. One cage was used for both the experiment and the control. When a control experiment was being made the air was shut off; no moving untreated air was used.

In the beginning, considerable difficulty was encountered in obtaining a constant flow of air through the apparatus and into the cages, due to the fact that the air supply was taken from the university air mains. The pressure in the mains ran about 85 pounds per square inch and as the experiments were conducted at a much lower pressure, from one to five pounds, a reducing valve was placed in the branch line to the apparatus, to reduce the pressure and keep it constant for any particular pressure that was required for the experiment on hand. On account of the well-known acute sensibility of man and mammals to slight differences in air movement it was thought best to pay considerable attention to air movement.

At the outset it was decided to obtain the flow of air in terms of a number of liters per minute as this can easily be determined. This was done by filling a 13-liter bottle with water and finding the time required for the air to displace the water. By proportion the flow in liters per minute was readily obtained but as the operation had to be repeated for each change in the air pressure the work for finding the flow for each different pressure was tedious and consumed much time, that it was decided to install some device, whereby the flow at any time could be readily obtained.

The subject of air in motion presents one of the most confusing branches of the study of the flow of fluids. The internal eddies, cross currents, and general intricacy of motion of the particles among each other, occurring in a pipe transmitting air, are almost entirely defiant of mathematical expression. It is necessary to adopt as a basis of mathematical investigation the simple assumption that the particles move side by side in such a way that those which at any instant form a lamina or thin sheet, perpendicular to the axis of the pipe or orifice, remain together as a lamina during the further stages of flow. Experiment is then relied upon to make good the discrepancies between the indications of the formula resulting from theory and the actual results of practice.

The laws governing the flow of all fluids are based on the assumption that the density remains constant throughout the flow, as has been stated above. In considering the flow of a gas such as air, however, the laws referred to do not strictly hold. The velocity in an air duct of uniform size varies due to a loss or decrease in pressure which causes an increase in volume and a consequent increase in the velocity. The flow of air, due to a large difference in pressure is most accurately stated by the thermodynamic formula for air discharge under conditions of adiabatic flow. The usual method in stating and measuring small pressures is by noting the height of a column of water which the pressure will maintain in equilibrium or balance in a "U" tube, or manometer.

From this we can analyze the different pressures acting when air flows through a pipe. The flow of air through a pipe or duct is under the influence of three distinct pressures, namely, the velocity, static, and dynamic or total pressures. The velocity head or pressure is defined as that pressure which is required to accelerate the mass from a state of rest to the final velocity attained. The static head or pressure, also termed the frictional or resistance pressure or maintained resistance, is that pressure required to overcome the resistance offered to the flow. This, in reality, is the pressure tending to burst the pipe as would be measured by the ordinary pressure gage. The dynamic head or pressure, also termed the total or impact pressure, is the sum of the pressures required to overcome the resistance to flow and create the velocity of discharge. For the many occasions where the measurement of volume, or of volume and pressure, is required, more accurate methods of measurement are often desired than have been commonly used, and the experimenter needs something better than the ordinary anemometer to determine the velocity, or a rubber tube connected to a water gage to measure the pressure. Especially is this so in fan work where the volume varies from zero with the outlet entirely closed, to a maximum, with an unobstructed discharge. The measuring apparatus must be accurate through this wide range.

As has been stated, the usual method employed in stating and measuring small pressures is noted by observing the height of a column of water which the pressure will maintain in equilibrium or balance in a U tube, or manometer. The difference in level of the liquid in the manometer is a measure of the static pressure existing at this point in the pipe or duct. A tube of this description is turned a "Piezometer." In order to obtain correct piezometer readings the most accurate method is to employ a hollow ring connected to the interior of the pipe by six or eight small holes, 0.02 inch in diameter.

Some form of differential or inclined tube gage filled with gasoline and graduated to read in hundredths of an inch of water is usually employed in place of the ordinary U tube, which is not well adapted for reading small pressure differences.

We can now measure the static head or pressure, but as this would not be of much value with air under motion, it is necessary to introduce some device in order that the velocity pressure may be obtained. Such an instrument is known as the "Pitot" tube. This Pitot tube when used with suitable pressure measuring devices, as already described, is particularly well adapted for measuring both the pressure and volume of air flowing through a pipe or duct.

The Pitot tube has the advantage of being small, portable, and can be easily and conveniently placed in small air lines. It is on

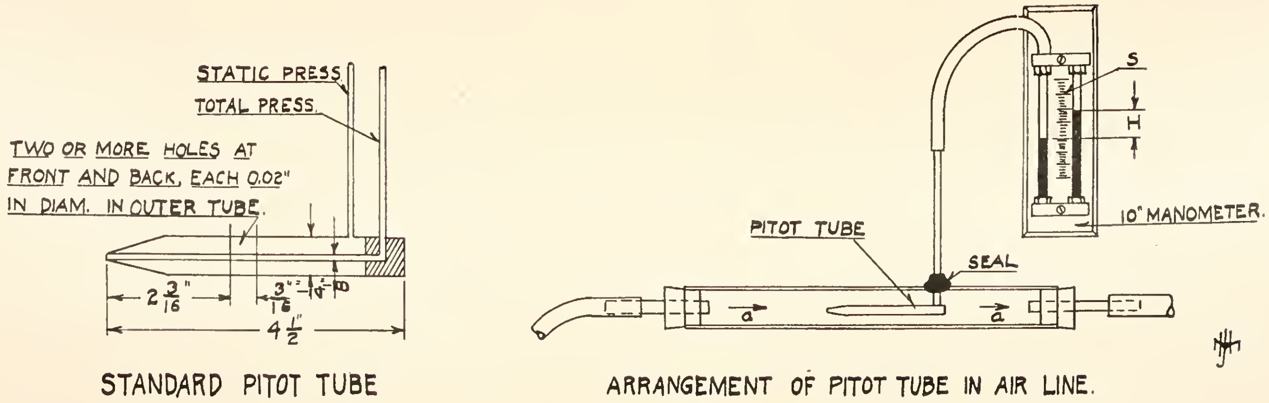
STATIC PRESS.
TOTAL PRESS.
TWO OR MORE HOLES AT FRONT AND BACK, EACH 0.02" IN DIAM. IN OUTER TUBE.
2 3/16"
3"/16 = 1/4" ID
4 1/2"
STANDARD PITOT TUBE
S
H
10" MANOMETER.
PITOT TUBE
SEAL
a
a
ARRANGEMENT OF PITOT TUBE IN AIR LINE.

FIG. 1. For description see text.

this account as well as on account of its small size that we describe it here though a better device is described by Hamilton ('17) in the paper which follows. It is a well-known instrument, and consists essentially of two parts, a tube pointing upstream against the flow of air or gas and which converts the sum of the static pressure and velocity pressure into a head which may be measured, and, as a second part, a means of determining the static pressure alone. Although the Pitot tube has long been known and used there is still much doubt in regard to its accuracy and distrust of the results obtained by its use. The form of tube shown in Figs. 1 and 2 has been generally adopted as being the most reliable.

The Pitot tube, as described, was sealed in a glass tube $1\frac{1}{4}$ inches in diameter and 30 inches long, and the connection made from the leads of the Pitot tube to a ten-inch mercury column manometer by means of a piece of one-half-inch rubber tubing. This general arrangement is shown in Fig. 1. After the apparatus had been set up as shown in the sketch the tube leading to the cage was inserted into the neck of the inverted 13-liter bottle and the air turned on. This was done in order that the scale *S* on the mercury manometer could be calibrated in rate of flow of the air. Several such readings were taken for the range of pressures to be used in the experiments, and a scale constructed whereby the rate of flow in liters per minute could be had directly by measuring the height of the mercury in the manometer. The rubber tubing from the exit side of the measuring device was then connected to the leads from the cages containing mice. Some of the later experiments were performed with the equipment described by Hamilton ('17).

2. *General Plan of the Experiments.*

The mice were put into the experimental cages and air of different evaporating power was secured by passing air of different relative humidities or different temperatures, or at different velocities through the different divisions of the cages. The bottom of the cage was covered with leaves and in order to keep the air from blowing them to one side melted paraffin was poured on them. This was done to procure a rough surface as the mice would not run over the smooth tin bottom. After the rate of evaporating power was determined the mice were put in the cage, and tracings to the minute and second scale were made for thirty-minute intervals.

A control experiment was made after each experiment so that a comparison could be made in the two cases. An animal might spend as much time in one division of the control cage as it did in the experimental cage but as the graph shows the movements are of a different character. There is no direct orientation in the control cage, and the mice wander back and forth through the different divisions, and seem to be as well satisfied in one division as the other. After one experiment was completed and the mouse had selected the division of the cage which seemed to agree with its physiological make-up, the different gradients were sometimes reversed. The mouse always moved out and sought the conditions it had formerly selected.

3. *Material.*

Only one species was used in the following experiments, the white-footed wood mouse, *Peromyscus leucopsus noveboracensis.* The experiments were made as soon as possible after the mice were brought into the laboratory, and until experiments were made they were kept under conditions as similar to those of their natural environment as was possible.

4. *Experimental Results.*

(*a*) *Reaction Experiments.*—The air was passed through sulphuric acid filters.[1] The amount of moisture in the air after treatment depended upon the rate of flow, the temperature, the original humidity of the air and the condition of the filters. These filters became weaker from time to time due to the water removed from the air. Ten-minute exposures were made of the atmometers, as this time was sufficient to calculate readable results. The rates are higher than they were in the experiments of Shelford ('13) as the flows were greater and the same atmometer containers were read. The evaporating results do not represent the rate in the cages for the cases were larger.

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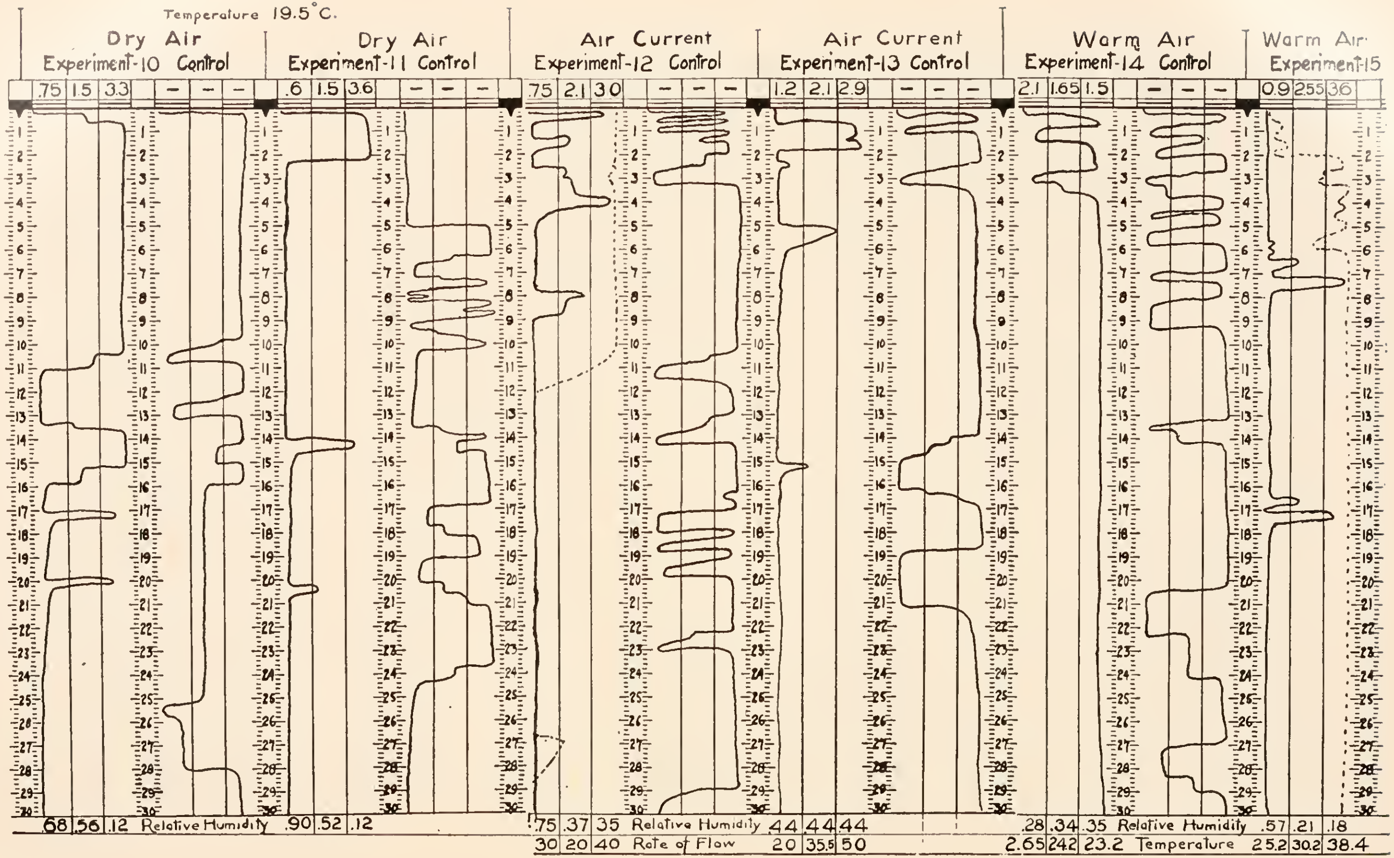
[1] Biol. Bull., No. 25, '13.



Temperature 19.5°C.
Dry Air
Experiment-10 Control
.75 1.5 3.3
Dry Air
Experiment-11 Control
.6 1.5 3.6
Air Current
Experiment-12 Control
.75 2.1 3.0
Air Current
Experiment-13 Control
1.2 2.1 2.9
Warm Air
Experiment-14 Control
2.1 1.65 1.5
Warm Air
Experiment-15
0.9 2.55 3.6
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
.68 .56 .12 Relative Humidity .90 .52 .12
.75 .37 .35 Relative Humidity .44 .44 .44
30 20 40 Rate of Flow 20 35.5 50
.28 .34 .35 Relative Humidity .57 .21 .18
2.65 24.2 23.2 Temperature 25.2 30.2 38.4

CHART I.

The chart shows the reactions of the white-footed wood mouse to air of various evaporative powers. The distance from right to left between the scales represents the length of the cage. The vertical scles are time scales with minutes divided into twelve second periods. The tracings represent the movements of the animals; horizontal distance represents the distance lengthwise of the cage and vertical distance represents time. The solid vertical lines separate the divisions corresponding to the thirds of the cage. The number at the heads of these columns in the experiments represent the evaporation in centimeters for the thirty-minute periods. The relative humidity and temperature data is given at the bottom of the columns. This chart shows a very marked avoidance of the air of high evaporation due to dryness. The lowering of the threshold of stimulation is evident in Experiment 10, as the period spent in the high evaporation conditions is less each time until direct orientation results. The mouse remained in this division of the cage forty-five minutes after the experiment was closed during which time it slept. In Experiment 11 the filters and moisteners were renewed. The avoidance of the air of high evaporation is noted but this mouse reacted much more definitely which was probably due to the dryer air and physiological state of the organism. The only time it left the air of lowest evaporation it was forced out by the experimenter. The figures at the top of the columns represent the evaporation in cubic centimeters during the thirty-minute experimental periods and those at the bottom represent the relative humidity.

The chart shows the negative reaction of a mouse to air of high evaporation due to increased air movement. In Experiment 12 the filters were removed from the air supply and the moisteners were left in. The mouse oriented directly to the air of highest evaporation, and the stimulation seems to be more of a mechanical nature but undoubtedly the direct orientation is a reaction to the higher evaporation due to the rapid air movement as well as the mechanical stimulus and loss of heat. At the close of the experiment the two ends were reversed. The dotted line represents the tracings of the animal's movements. The same avoidance of the air of high evaporation is noted but the mouse did not orient directly. It remained in the unfavorable conditions for ten minutes but became very restless after the second minute and finally moved to the air of the same evaporation it had previously selected. At the end of twenty-seven minutes it was forced out by the experimenter but returned immediately to the air of lowest evaporation. In Experiment 13 the moisteners were removed. When the mouse first encountered the rapidly moving air it was stimulated and kept turning around in the cage and displayed other random movements. After returning to the air of lowest evaporation and the less rapid air movement the turnings were replaced by hesitation at the boundary of the highest evaporation rates and shorter stays in the rapidly moving air.

The chart shows the avoidance of high evaporation due to increase of temperature. In Experiment 14 a difference of 3.2° C. was noted. The standard rate of flow, 27 liters per minute, was used in this experiment. In Experiment 15 the orientation to the higher temperature is more direct, and by comparing the two experiments it is seen that temperature differences are easily detected by these mice. The dotted line represents the tracings of the animal's movements when the ends were reversed at the end of the experiment. The mouse ran around at random for the first five minutes and then selected the air of the same evaporation it had previously selected.

About thirty experiments were performed to test the reactions of these mice to the different evaporating powers of the air. The preceding graphs of a few of the experiments will serve to show the general behavior of these mice in all the experiments.

In Experiment 10, Chart I., the moisteners were becoming very dry and the filters were weak; both were renewed in Experiment 11. The standard rate of flow, 27 liters per minute, was used in both experiments. The mouse was always placed in the division of the cage where evaporation was the lowest. In Experiment 10 the mouse moved directly to the opposite end of the cage where the rate of evaporation was the highest and remained there ten minutes. It behaved quite normally for five minutes, then it grew restless and kept turning around in the corner of the cage; several times it stretched and lay flat and yawned. After moving back to the division of lowest evaporation it made three trips across the cage but the period spent in the dry air was less each time. The threshold of stimulation was lowered each time so that finally the mouse oriented directly, and returned to the air of low evaporation. Theoughout the rest of the experiment it remained there and behaved normally. Thirty minutes after the experiment was closed the mouse was still in the same division of the cage. In Experiment 11 the mouse reacted much more definitely, due in part no doubt to the air of higher evaporation and the physiological state of the animal. It remained in the air of lowest evaporation throughout the experiment excepting when it was forced out. A marked negative reaction to the highest evaporation is clear in both experiments.

The rate of flow influences the rate of evaporation in a marked degree. The mice oriented directly in most cases to a very small change in the rate of flow, but I am inclined to attribute this orientation more as a response to a mechanical stimulation; however the higher rate of evaporation was no doubt a factor in determining this response. In their natural environment changes in wind velocity would directly influence their behavior though these variations are very slight in the forest. In their underground burrows it would have little or no effect. The differences in wind velocity existing between the forest and the prairie no doubt play an important rôle in restricting their habitats to the forest. The rate of evaporation is much greater on the prairie due to a more direct effect of the wind and a higher temperature.

In Experiment 13, Chart I., the rates of flow were 20, 35.5 and 50 liters per minute respectively; the filters and moisteners were both cut out of the air supply leading to the experimental cages. While most of the time was spent in the division representing the lowest evaporation the mouse never behaved normally; the lowest evaporation appearing to be above the optimum, and no doubt it was much greater than that encountered in the natural environment. In Experiment 12, Chart I., the rates of flow were 30, 30, and 40 liters per minute. The filters were cut out in this experiment while the moistener were left in, which accounts for the variation between the two 30-liter flows. The mouse showed a marked avoidance to the more rapid rate of flow. At the end of fourteen minutes the mouse was asleep in the division of lowest evaporation and remained there throughout the experiment. At the end of this experiment the ends were reversed. The mouse up to this time had been asleep but was now stimulated and after the first few minutes grew very restless, turning around in the corner and showing various other stimulation reactions similar to those described under the dry-air experiments. The mouse after ten minutes had elapsed moved to the end of lowest evaporation which it had previously selected and remained there throughout the experiment except at the end of the 27th minute, when it was forced out by the experimenter.

Only a general idea of the effect of raising the temperature can be obtained as the apparatus for this part of the experiments was faulty. It was impossible to keep the temperature constant, and the atmometers and the water in the burettes should have been at the same temperature as the air used, if the results are to be comparable with those obtained at room temperature. Even though the data may not be accurate the number of experiments performed give a fair indication of the general effect upon the organism as is indicated by the general behavior of the mice in all the experiments.

The various temperature gradients were obtained by passing the air through coils of aluminum tubes exposed to steam. In some cases the pressure exerted on the air in these coils was sufficient to raise the temperature 0.5° C. or more and thus produce a gradient. The temperature of the different divisions of the cage was recorded when a constant temperature seems to prevail. Thermometers reading to one tenth of a degree were used.

In Experiment 14, Charts I., the standard rate of flow, 27 liters per minute, was used, and the temperature in the different thirds were 26.5°, 24.2°, and 23.2° respectively. The reactions to the air of highest evaporation was similar to those of the dry and rapidly moving air, and in this experiment the mouse easily detected a difference of 3° C. In Experiment 15 the temperatures were 25.2°, 30.2°, and 38.4° respectively while the rate of flow was approximately 22 liters per minute. The dotted line represents the reaction of the mouse after the ends were reversed at the end of the experiment. The mouse was stimulated at first in the hot air; activity was increased and it moved back and forth in the cage for the first few minutes, after which it selected the same air as before.

(*b*) *Resistance Experiments.*—The other experiments show that the mice react to conditions of high evaporation. The following were performed in order to determine how long they could withstand these unfavorable conditions. The standard rate of flow used was the same as in previous experiments and the air was dried and moistened as previously described. The mice were put into large-mouthed bottles fitted with inlet and outlet tubes. As soon as the air was turned on the temperature and relative humidity were recorded.

Three experiments were started at the same time. In one bottle the mouse was treated with air of 15 per cent. relative humidity and with an evaporating power of 1.3 c.c. All evaporation readings are given for ten-minute readings. It died after a 41-hour treatment. In the second bottle the mouse was treated with air of 95 per cent. relative humidity and the evaporation was .3 c.c. The experiment was discontinued after 50 hours. The mouse had acted normally throughout the experiment. In the third bottle the mouse was treated with air that was warmed. The humidity was 29 per cent. and the evaporation .7 c.c. Death resulted after a 50-hour treatment. In the warm air and the dry air treatment the behavior of the mice was very similar.

After a few hours' treatment the mouse in the dry air became very active. Beginning at the end of 16 hours it was quiet again for several hours and it then became active again. It would run up and seize the outlet tube with its teeth holding on until shaken loose. In a few minutes it would repeat the performance. After 36 hours it was very weak and was in a comatose condition; it died 5 hours later after 41 hour exposure.

Three mice were treated with air of different rates of flow. The rates in liters per minute were 13, 30.5, and 45, and the rates of evaporation were .35 c.c., .7 c.c., 1.7 c.c. respectively. The mouse in the 45-liter flow died after a 19-hour treatment. Another mouse was put in the same bottle and weighed accurately. It died after a 16-hour treatment and lost 2.69 grams. The mice died with symptoms similar to the salamanders and frogs from dense woods, tested by Shelford ('13) but the time to death was much longer because of the covering of hair; and they are not so restricted to their habitat, as they are sometimes found living up in trees where the rate of evaporation is much greater than on the ground.

5. *General Discussion.*

Whether the evaporation was increased by more rapid rate of flow, by drying the air, or by raising the temperature the general behavior and reactions of the mice were the same. As a rule the mice did not orient as quickly in the dry air as they did to a difference in the rate of flow, or under certain conditions a change in temperature. But differences were far less in the case of mice in temperature gradients than in the case of Shelford's animals; where the evaporation was the same there was no difference. The mouse was always stimulated in the dry air and showed agitation by rapid turnings in the cage, yawning or by stretching and lying flat on the bottom. Sometimes the mouse moved about at random in the division of the cage representing the highest evaporation. This was especially the case when the ends were reversed after the completion of the experiment (Experiments 12 and 15). The selection of the air of lower evaporation was usually accomplished by the mouse moving back and forth along the cage; each period spent in the higher evaporation lowered the threshold of stimulation and finally avoidance resulted. The problems involved in the results of these experiments are complex and the rate of evaporation plays an important rôle in the distribution of animals.

Why is the habitat of the white-footed mouse coextensive with the woodland area or why is any animal restricted to any particular environment? If any one should attempt to answer these questions he would encounter a very complex physiological problem; however it is evident that this experiment work incates that the evaporation is the best index of conditions affecting warm-blooded animals.

Change of environmental factors influence organisms in one of three ways (Shelford '14): (*a*) They may produce death. (*b*) They may modify structure or behavior. (*c*) They may stimulate migrating animals and cause them to turn back when an increase or decrease in the factor is noted. These factors have been shown to modify form, color, size and the behavior of animals under experimental conditions, Entemann ('09), (Allen '74) and Sumner ('10).

The integument or covering of an animal has a great deal to do with the power to withstand air of high evaporation. The white-footed mouse with a heavy coat of hair no doubt can withstand these unfavorable conditions more readily than the salamander or other soft-skinned amphibians, since there is no mechanism to prevent loss of water. The shrew (*Blarina brevicauda* Say) has thinner coverings of hair and they live in a much more restricted habitat. It is almost impossible to keep them in the laboratory. The essential factor for their existence seems to be plenty of moisture and they will die if left in the traps over night without water.

When the mice encountered the air of high evaporation there was first a period of stimulation or of heightened sensibility which is indicated by the increasing avoidance of the air of high evaporation after several entrances into it as shown in all the experiments. It is impossible to give a solution which would account for this heightened sensibility, but the work of past investigators gives some interesting data on which to base some conclusions. Waller ('09) showed that a small increase of CO_2 increased the irritability of the nerves. Osborne ('10) showed that CO_2

output increased in dry air. The following table will make this clear.

Date.	Dry Bulb.	Wet Bulb.	Water Loss in Grams.	Co_2 per Hour.
February 8	27.1° C.	15° C.	85 c.c.	21.26
February 13	27.1° C.	12.5° C.	94.8 c.c.	23.78

Increased irritability in the mice after a few invasions of the dry air which caused them to turn back more quickly (See Graph 1) was probably due to increased CO_2 production. The differences in water loss and CO_2 given off is no doubt due to the varying humidity and velocity of the air. He showed that the increased water loss means an increased heat loss and therefore increased metabolism if the body of the organism is to remain thermostatic; that the ventilation of the lung is increased when the air temperature is high; that CO_2 varies directly and not inversely as the external temperature. These results show the importance of humidity cannot be overestimated, for temperature data without the known humidity is of no practical value.

It is evident to naturalists that mammals select certain local habitats for their chief places of abode and that their geographic distribution is a function of the distribution of the conditions so selected. In spite of many theories as to the factors controlling distribution of mammals there are apparently no experiments showing the factors to which they react in selecting their habitats. The experiments performed show that the moist forest animals studied avoid air of high evaporating power, due to dryness, rapid movement or high temperature. The negative reactions to air of high temperature which gives the same amount of evaporation as dry air is no sharper than in the case of dry air with no difference in temperature.

The experiments indicate that in the case of the mammals studied the rate of evaporation is more important than temperature in determining their movements. Their resistance to water withdrawal is far greater than that of any other animal in the same community.

IV. SUMMARY.

1. The mice reacted to evaporation whether it was produced by movement, dryness or heat (p. 192, 194–195).

2. The negative reactions to air of high temperature which gives the same amount of evaporation as dry air is no sharper than in the case of dry air with no difference in temperature (p. 192, 196).

3. They behaved normally in air of low evaporation, their optimum, but when the evaporation was increased the mouse was stimulated and reacted negatively (p. 192, 194).

4. Slight differences in air movement were easily detected by the mice (p. 195).

5. The rate of evaporation is the best index of the combined action of wind, temperature, and dryness of the air.

6. The integument of animals is important in determining the rate of evaporation, and animals in the same community show differences in their ability to withstand unfavorable conditions. The hair of the mouse makes it nearly as resistant as the heavily chitinized insects.

7. Evaporation is probably the most important factor in determining the distribution of the white-footed wood mouse.

V. Acknowledgments and Bibliography.

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THE OSMOTIC CONCENTRATION OF THE SAP OF THE LEAVES OF MANGROVE TREES.[1]

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I. Problem and Methods.

The morasses of insular and continental coasts in the tropical zone of both eastern and western hemispheres are largely occupied by a vegetation consisting almost exclusively of a number of species of trees or shrubs collectively designated as mangroves.

These species, belonging to different genera and families, are characterized by striking morphological and biological features, for example, the prop or stilt roots of *Rhizophora*, the negatively geotropic roots, breathing roots or pneumatophores of *Avicennia*, and the remarkable vivipary and seedling dispersal of *Rhizophora*.

These various peculiarities have attracted the universal interest of biological travelers in the tropics.

The physiology of a group of organisms characterized by such morphological features and growing under environmental conditions so specialized presents a most interesting group of problems. When, however, one turns to the literature he is impressed by the fact that conclusions concerning the physiology of these plants are chiefly deductions from all too superficial field observations and from macroscopic and microscopic anatomical investigations. Physiological constants, properly so called, are practically wanting. Thus the recording of any quantitative data on the physiology of this ecologically fascinating group of plants is especially desirable. In the present paper we shall not summarize or comment upon the available literature, but shall limit ourselves to the presentation of the results of certain measurements carried out on the southern shore of the island of Jamaica in the winter of 1915 and in the Everglades region of southern Florida in the winter of 1916.

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[1] Coöperative studies carried out by the Department of Experimental Evolution and the Department of Botanical Research of the Carnegie Institution of Washington.

The specific problem attacked is that of the osmotic concentration of the tissue fluids.

It is quite natural that zoölogists should have led in the investigation of the relationship between the concentration of the tissue fluids and of that of the medium surrounding the organism. They have had at their disposal a wealth of forms exhibiting the widest possible range of organization, living in a medium which while showing a considerable range in concentration is nevertheless relatively constant over large areas, and opposes no obstacles to the easy extraction of samples of fluids (blood) for comparison with the medium. Among those who have contributed to the literature of this field may be mentioned Botazzi, Fredericq, Garry, Macallum, Green, Dakin, Mosso, Dekhuyzen, Scott, and others. The subject is discussed by R. Höber in his "Physikalische Chemie der Zelle und der Gewebe." Just as these pages were being prepared for the printer a summary by Scott ('16) of the data available for the depression of the freezing point of the blood of representatives of the various phyla of the animal kingdom, with the values obtained for the water in which the individuals were taken, appeared.

The problem of the osmotic concentration of the cell sap of the plant organism in relation to its medium has been rather extensively studied by a number of workers on the algæ and fungi. In recent years a number of investigations have also been carried out on the higher plants, both in the laboratory and in the field. These botanical studies need not be reviewed here.

It is perhaps clear that a study of arborescent plants growing in the saline substratum of the tropical coast has certain points of interest over that of the investigation of algæ or other submerged organisms. Not only is there the factor of the osmotic pull necessary in water ascent but there is the possibility of the increase in osmotic concentration of the foliar tissue fluids by the retention in solution (and hence in an osmotically active condition) of the salts absorbed from the concentrated substratum and left in the leaf tissue by the rapid transpiration incident to tropical temperature, insolation and air movements.

The purpose of this paper is to present the results of a series of determinations of the concentration of the leaf tissue fluids of the three genera, *Avicennia, Rhizophora* and *Laguncularia.*

This has been expressed in terms of molecules and ions as measured by the freezing point lowering Δ, corrected for undercooling (Harris and Gortner, '14), and in atmospheres pressure P as given in the paper just cited and in a supplementary table (Harris, '16) necessitated by the high concentrations found in the leaf tissues of the mangroves of the swamps on the southern shore of Jamaica and in the plants of the adjacent coastal desert (Shreve, '10). All determinations were based on sap extracted from tissue previously frozen (Gortner and Harris, '14), to increase its permeability, as emphasized by Dixon and Atkins ('13) and ourselves ('16). On approaching the freezing point or in passing it in undercooling, the sap of all three species generally shows a cloudiness or precipitation as noted by Gorke ('06). That this sensibly influences the freezing point lowering seems rather improbable, but we have not been able to give the subject adequate investigation.

Some difficulty was experienced with slight or heavy salt incrustations which sometimes occurred on the leaves, and are not easily removed without washing. To what extent this is of internal origin we are not able to assert. Probably it does not appreciably influence the constants upon which our discussions are based. Two determinations based on leaves of *Avicennia* from Port Henderson, Jamaica, which had particularly heavy incrustations, which were probably not thoroughly removed, gave:

Mar. 30, $\Delta = 5.86$, $P = 70.0$; April 2, $\Delta = 5.86$, $P = 70.0$.

II. HABITATS FROM WHICH COLLECTIONS WERE MADE.

The mangroves at Port Henderson form a dense thicket in a low area back of a gravelly, rather sterile beach which separates the swamp proper from the open sea. The vegetation consists of a dense growth of *Avicennia nitida* Jacq. with a few specimens of *Rhizophora mangle* L. and *Laguncularia racemosa* (L.) Gaertn. f. The pneumatophores of *Avicennia* thickly studded the water-covered soil or soft mud in most of the swamp.

The water here was not abnormally saline. One sample taken near the stilt roots of the collection of *Rhizophora* made January 22 gave $\Delta = 2.05$, $P = 24.7$. Another sample taken near the pneumatophores of *Avicennia* gave $\Delta = 1.96$, $P = 23.5$.

These determinations are in excellent agreement with those given by Garry ('15) for sea water from southern localities. Among his maximum values are $\Delta = 1.90$–1.93 for Pacific Grove, Cal., $\Delta = 2.04$ for Beaufort, N. C., and $\Delta = 2.24$ for Naples.

Back of the swamp which fringes a small bay with water too deep for the growth of either of the species, is a considerable area of almost sterile mud flat. Here the soil solution must become highly concentrated by evaporation from the superficial soil layers of the water left by occasional tidal overflow. We had no means of measuring this, but a sample of water from a slight depression, possibly diluted by a recent shower, gave when frozen $\Delta = 5.77$, $P = 68.9$. The mangroves occur only on the edges of these mud flats. Practically the only other species found here are the succulent-leaved halophytes, *Batis maritima* and *Sesuvium Portulacastrum*.

The determinations from subtropical Florida were based on plants growing on the mainland shore of Biscayne Bay at Miami and Cocoanut Grove and on the Everglades or Front Prairie, as some term it, south of Florida City.

A single sample of Biscayne water taken near the shore at Cocoanut Grove gave $\Delta = 1.45$, $P = 17.4$. This determination is distinctly lower than those cited for the seacoast localities, and more nearly comparable with the $\Delta = 1.09$ for Kiel Harbor, $\Delta = 1.30$ for the open Baltic sea, or $\Delta = 1.66$ as determined in the Kattegat by Dakin and cited by Garry (*loc. cit.*).

The dwarfed mangroves growing on the Everglades near Florida City are in a practically non-saline substratum. A sample of water from a ditch froze at $-.009°$, a value in good agreement with that for bog and pond water as studied by Livingston ('04) and Transeau ('16).

Scott ('16) classifies water with a depression of 0.03° as fresh.

III. Presentation of Constants.

A. *Avicennia nitida* Jacq.

The leaves of trees of *Avicennia nitida* from the Port Henderson swamp proper gave:

Jan. 20, $\Delta = 4.26$, $P = 51.1$
Jan. 22, $\Delta = 3.29$, $P = 39.4$
Jan. 22, $\Delta = 3.56$, $P = 42.7$
Mar. 26, $\Delta = 3.47$, $P = 41.5$
Mar. 30, $\Delta = 3.60$, $P = 43.2$
April 2, $\Delta = 3.84$, $P = 46.0$

Average, $\overline{\Delta} = 3.67$, $\overline{P} = 43.98$

Plants from the edges of the sterile mud flats gave the following values:

Jan. 20, $\Delta = 3.67$, $P = 43.9$
Jan. 25, $\Delta = 4.55$, $P = 54.4$
Mar. 26, $\Delta = 3.90$, $P = 46.7$
April 2, $\Delta = 4.29$, $P = 51.3$

Average, $\overline{\Delta} = 4.10$, $\overline{P} = 49.08$

Thus on the southern coast of Jamaica *Avicennia nitida* is characterized by leaf sap showing a concentration of about 45 to 50 atmospheres. In view of the considerable variation in the individual determinations it is impossible to assert that the concentration in the plants growing on the edge of the sterile mud flats is *significantly* higher than that of those occurring in the swamp proper.

For the most part, the leaves taken were fully mature, but on Jan. 20th, it was possible to secure some new-growth leaves in the collection from the mud flats. These gave $\Delta = 3.56$, $P = 42.6$ as compared with $\Delta = 3.67$, $P = 43.9$ for the old leaves. Thus the old and the young organs show about the same concentration of tissue fluids.

Two collections of the leaves of seedlings, about 1–2 dm. in height, growing in mud or shallow water in the rather dense shade of the trees gave:

Jan. 20, $\Delta = 4.47$, $P = 53.5$
Jan. 22, $\Delta = 4.02$, $P = 48.1$

Note that these values are actually slightly in excess of those determined from the leaves of the trees collected on the same dates.

A tree of *A. nitida* about 16 cm. in diameter on the mainland shore of Biscayne Bay at Miami gave:

Feb. 24, $\Delta = 2.76$, $P = 33.1$

This constant is distinctly lower than any of those determined at Port Henderson, and indicates that the latter values are influenced by the growth of the plants under the general environmental conditions which have resulted in the development in this region of the coastal desert flora (Harris and Lawrence, '17).

B. Rhizophora mangle L.

Rhizophora was not abundant on the Jamaican coast where our collections were made. The shrubs occurred only in the swamp. The constants determined were:

Jan. 22, $\Delta = 2.47$, $P = 29.6$
Jan. 25, $\Delta = 2.57$, $P = 30.9$
Mar. 26, $\Delta = 2.43$, $P = 29.2$
Mar. 30, $\Delta = 2.53$, $P = 30.4$
April 2, $\Delta = 2.49$, $P = 29.9$

Average, $\bar{\Delta} = 2.50$, $\bar{P} = 30.0$

Small trees of *R. mangle* on the mainland shore of Biscayne Bay yielded leaves which gave the following concentrations:

Feb. 8, $\Delta = 2.20$, $P = 26.4$
Feb. 17, $\Delta = 2.24$, $P = 26.9$
Feb. 24, $\Delta = 1.95$, $P = 23.4$

Average, $\bar{\Delta} = 2.13$, $\bar{P} = 25.57$

On the prairie below Florida City *Rhizophora* grows in a substratum where the amount of salt in the soil must be insignificant. Here the constants were found to be:

Feb. 18, $\Delta = 1.85$, $P = 22.2$
Feb. 29, $\Delta = 1.84$, $P = 22.1$
Feb. 29, $\Delta = 1.91$, $P = 22.6$

Average, $\bar{\Delta} = 1.87$, $\bar{P} = 22.30$

The reader will have noticed three salient results in these constants. First, the concentrations in *Avicennia* are distinctly higher than in *Rhizophora*. Second, that the Florida collections give distinctly lower constants than do those made in Jamaica. Third, that there is a distinct reduction in the osmotic concentration as the Florida plants leave the shores of the bay and penetrate into the non-saline substratum of the Everglades region.

C. *Laguncularia racemosa* Gaertn. f.

At Port Henderson, *Laguncularia racemosa* occurs both in the swamp and on the relatively sterile mud flats.

In the swamp the trees gave:

Jan. 22, $\Delta = 2.07$, $P = 24.9$
Jan. 22, $\Delta = 2.05$, $P = 24.6$
Mar. 26, $\Delta = 2.13$, $P = 25.6$
Mar. 30, $\Delta = 2.14$, $P = 25.7$

Average, $\bar{\Delta} = 2.10$, $P = 25.20$

On the relatively sterile mud flats the concentrations were:

Jan. 20, $\Delta = 2.90$, $P = 34.8$
Jan. 25, $\Delta = 2.90$, $P = 34.7$
Mar. 26, $\Delta = 2.79$, $P = 33.5$
Mar. 30, $\Delta = 2.69$, $P = 32.3$
April 2, $\Delta = 2.75$, $P = 33.1$

Average, $\bar{\Delta} = 2.81$, $\bar{P} = 33.68$

While it was not possible to assert that the osmotic concentration of the leaf sap of *Avicennia* was significantly higher when growing on the sterile mud flats, there can be no reasonable question of the relative values of the constants in the case of *Laguncularia*. The five individual values from the flats are without exception higher than the four available from the swamp.

Two large trees of *L. racemosa* on the mainland shore of Biscayne Bay gave:

Miami, Feb. 17, $\Delta = 2.11$, $P = 25.3$
Cocoanut Grove, Feb. 26, $\Delta = 2.24$, $P = 26.9$

Young trees about 1½ m. high gave:

Miami, Feb. 24, $\Delta = 1.81$, $P = 21.7$
Cocoanut Grove, Feb. 26, $\Delta = 1.73$, $P = 20.8$

That the sap of *L. racemosa* is profoundly influenced by its substratum is splendidly shown by a determination based on trees growing in practically fresh water on the south shore of Jamaica. An irrigation canal passes through the coastal desert. Trees[1] growing in the marsh beside it gave:

Jan. 30, $\Delta = 1.64$, $P = 19.7$

IV. RECAPITULATION.

The present paper is a contribution to the problem—hitherto practically untouched by quantitative methods—of the physiology of mangrove vegetation. Specifically it deals with the osmotic concentration or osmotic pressure of the leaf tissue fluids.

Three species, *Avicennia nitida* of the Avicenniaceæ, *Rhizophora mangle* of the Rhizophoraceæ, and *Laguncularia racemosa* of the Terminaliaceæ have been examined on the southern shore of the island of Jamaica and in southern Florida. Determinations of freezing point lowering of sap extracted from plants growing in habitats ranging from a substratum saturated with fresh water to the margins of highly saline and practically sterile mud flats have been secured.

The concentration of the tissue fluids is relatively high throughout. The minimum concentrations are about 20–22 atmospheres for *Laguncularia* and *Rhizophora* growing under practically fresh-water influence and 33 atmospheres for *Avicennia* on the shore of Biscayne Bay. The values may range widely in response to local conditions. Thus on the southern coast of Jamaica where the plants are growing under the influence of the edaphic and meterological conditions which on higher levels have resulted in a splendidly developed desert vegetation, *Avicennia* shows trustworthy measurements of concentrations up to fifty atmospheres in leaves in a fairly healthy condition. It is quite probable that somewhat higher values will be found.

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[1] Determinations on three more or less herbaceous forms were secured for comparison

Ammania latifolia L. April 2, $\Delta = 1.60$, $P = 19.3$
Pluchea purpurascens (Sw.) D.C. April 4, $\Delta = 1.21$, $P = 14.5$
March 30, $\Delta = 1.25$, $P = 15.0$
Verbesina alba L. (*Eclipta alba* (L.) Hassk.) April 12, $\Delta = 1.60$, $P = 19.2$

Rhizophora mangle shows concentrations of about 25 atmospheres on the mainland shore of Biscayne Bay and 30 atmospheres in the swamp at Port Henderson, Jamaica, as compared with slightly over 22 atmospheres under the practically fresh-water influence of the Everglades. *Laguncularia racemosa* shows a concentration of about 20 atmospheres under nearly fresh-water influence at Port Henderson, from 20 to 25 atmospheres on the shore of Biscayne Bay, about 25 atmospheres in the mangrove swamp and about 34 atmospheres on the edges of the sterile mud flats near Port Henderson.

Apparently *Avicennia* is capable of developing a much higher sap concentration than either of the other species investigated. Thus on the shore of Biscayne Bay it showed a concentration of about 33 atmospheres, whereas the maximum values for *Rhizophora* and *Laguncularia* were about 27 atmospheres. In the swamp at Port Henderson the leaves of trees of *Avicennia* gave a freezing point lowering indicating a concentration of about 44 atmospheres as compared with 30 atmospheres for *Rhizophora* and about 25 atmospheres for *Laguncularia.* Even the leaves of seedling plants of *Avicennia* exhibited a concentration of about 50 atmospheres as compared with the values just cited for adult leaves of *Rhizophora* and *Laguncularia.*

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BIOLOGICAL BULLETIN

EXPERIMENTAL ALTERATION OF THE AXIAL GRADIENT IN THE ALGA, GRIFFITHSIA BORNETIANA.

C. M. CHILD.

(WITH 15 FIGURES.)

The existence of axial gradients in susceptibility to various agents and conditions has been demonstrated as a characteristic feature of axiate animals, and the relation of such gradients on the one hand to metabolic and protoplasmic condition and on the other to the morphological and physiological order or pattern characteristic of development has been discussed in various publications.[1] The results of these investigations on animals led to the attempt to determine whether similar axial gradients occur in plants and some of the data on algæ have already appeared (Child, '16*c*, '16*e*, '17). The existence of such gradients in the axes of at least certain plants as well as in the axes of animals being demonstrated, it remains to determine their relation to the order or pattern of the plant axis. To accomplish this completely it would be necessary to obliterate or reverse existing gradients, to determine experimentally the origin of new gradients and to observe the effect of such changes upon order or pattern in the plant body.

Obliteration and reversal of axial gradients in algæ by various means have already been described (Child, '16*e*, '17). In most cases these changes represented a stage in the process of dying, and while they showed that changes might occur, the fact that these changes were soon followed by death made it impossible to determine their effect upon the developmental and morphological order in the plant body. In the present paper some experimental alterations of the gradient which do not necessarily end in death are described, and some evidence concerning the effect of these changes on further development and axiation is presented. This is merely the first step toward the experimental determination of the relation between the axial gradient and polarity in the plant and the very simple experiments described here serve chiefly to point the way to more exact determination and control.

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[1] See Child, '15*a*, Chap. IX., '15*b*, '15*c*, '16*a*, '16*b*, '16*c*, '16*d*. Hyman '16, and references to further literature in these publications.

For experimental purposes a form with large cells and sensitive enough to react readily to environmental changes but not too sensitive was desirable. I am indebted to Professor Osterhout for calling my attention to *Griffithsia*, which has proved to be of interest in various ways.

The Griffithsia Axis and Gradient.

The cell outline of an average, well-developed vegetative axis and branches from the material employed is indicated in Fig. 1, the hairs (Child, '17) being omitted. The method of growth is evident from the figure. The apical cell grows to a certain variable size and divides into a very small apical cell which repeats the process of growth and division, and a larger subapical cell, which continues to grow, but does not divide unless it later gives rise to a new axis by the separation of a new apical cell from some point of its apical region, as in the formation of new branches (Fig. 1). At a greater or less distance from the apical end a secondary basal elongation of the cells begins and is evident in the progressive change of shape of the cells toward the basal end of the axis (Fig. 1).

As already noted (Child '16*e*), the general susceptibility gradient in such an axis and in each branch is basipetal when the plant is in good condition. In the apical third or half of the length the gradient is very uniform, but in the basal region irregularities occur consisting in a susceptibility in one or more cells above or below the expectation corresponding to the level of the cell or cells. In the two or three most apical cells the intracellular gradient is usually slight or sometimes not visible, but is basipetal when present. Often the apical cell itself shows a distinct basipetal gradient. Several cells from the third or fourth on usually show a distinct basipetal intracellular gradient, but with the beginning of the secondary basal elongation of the cell a basal region of high susceptibility usually appears, and some of these elongating cells show a double gradient, a region near the middle being the least susceptible. In these elongated cells of the more basal levels of well-developed axes the intra- cellular gradients may differ from cell to cell and from time to time, even though the general axial gradient remains essentially unaltered.

Not infrequently rhizoids develop on these more basal elongated cells even at a distance of several cells from the basal end of the axis, and in such cases the rhizoid may appear at any level, even close to the apical end of the cell, while the latter is still, at least physically, a part of the axis. In cases where such well-developed rhizoids are found on such cells it is of course impossible to determine the relation between the intracellular gradient and the point of origin of the rhizoid at the time when the rhizoid arose, and the possibility always exists that the intra-cellular gradient may have undergone alteration since the origin of rhizoid. Rhizoid development is primarily a feature of the basal end or the more basal regions of the axes of algæ (Tobler, '06), and as such we might expect to find it associated with regions of low susceptibility, *i. e.*, of low metabolic rate. The alterations in the intracellular gradients of the older more basal cells of *Griffithsia* suggest the possibility that even where a rhizoid arises from the middle or the apical region of such a cell this region may be the region of lowest metabolic rate in that particular cell.

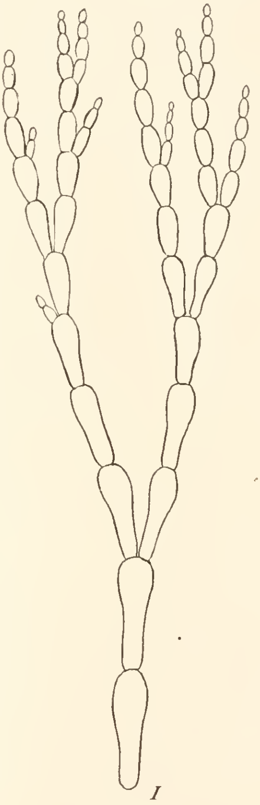


Fig. 1.

The appearance of rhizoids or rhizoid-like structures on the older, more basal parts of the axes, is a characteristic feature of various species of algæ and in some cases these structures are so numerous as to form a more or less continuous cortical layer on such parts. In *Dasya*, for example, where such a secondary cortex formed by rhizoids is present on the older portions of the axes, the cells of this region show a relatively low susceptibility. In short, the rhizoid appears to be a structure originating at the lower levels of an axial metabolic gradient, though of course external conditions may determine whether or not rhizoids shall develop at levels where their development is possible.

Some of the experimental evidence presented below, as well as the alteration and reversal of the primary intracellular gradient in the more basal cells indicates very clearly that the degree of axiate individuation, *i. e.*, the permanence of the primary metabolic axial relations and their effectiveness in maintaining a particular order is not very high. Moreover, the short length which the axes attain, the slight connection between the cells, the irregularity of branching and the above-mentioned development of rhizoids from any level of the more basal cells all support this conclusion.

If physiological polarity is primarily a metabolic gradient in protoplasm, such a form as *Griffithsia* should be favorable material for the experimental obliteration of old axes or gradients and the establishment of new ones. The experiments described below, which are suggestive rather than conclusive, constitute a first step in this direction.

Experimental Obliteration and Reversal of the Gradient in Relation to Individuation.

The fact that obliteration or reversal of the general axial gradient of *Griffithsia* usually occurs during the first few days in the laboratory, even where the plants are kept in running water, has been noted in an earlier paper (Child, '16*e*). The results of a few simple experiments along this line remain to be considered.

Confinement in a limited supply of water without change in a closed flask without air was used as an extreme degree of "bad" environmental conditions. In these experiments masses consisting of many axes were placed in 100 c.c. Erlenmeyer flasks which were filled with water, corked without air space and kept in the running water of the laboratory aquarium under the same conditions of light and temperature as the stock.

Susceptibility tests were made on the fresh material before confinement, and at intervals during the experiment the susceptibility to KCN *m*/100 or *m*/50 of portions removed from the flask was compared with that of controls from the same plants kept in running water, the phycoerythrin serving as indicator of death, as described in an earlier paper (Child, '16*e*).

The results of these experiments are briefly as follows: the plants of course die in the flask in a few days at most, in my experiments in 2–7 days, the survival time varying in general inversely as the amount of plant tissue present. The death gradient is basipetal as with other agents, *i. e.*, the apical regions are most susceptible to the confinement and the susceptibility decreases basipetally. The progress of death can be observed with the naked eye without difficulty, for each cell as it dies becomes orange-yellow by reflected light.

After twenty-four hours' confinement and before the death of any cells the susceptibility to KCN has decreased and the general susceptibility gradient in KCN shows a more or less complete reversal in the apical regions of most axes. This reversal may involve only the apical cell itself, in which case it dies later instead of earlier than the cell next below it, or it may include two, three or even the five or six most apical cells of the axis. In such cases these cells are less susceptible and die later than the cells next below, and in this group the death gradient from cell to cell is more or less distinctly acropetal, the apical cell dying last. Where the apical cell has recently divided and is very small it may still show a high susceptibility and the reversal may occur in the subapical region.

After forty-eight to sixty hours' confinement the susceptibility to KCN is usually again as high as or even considerably higher than that of fresh plants and the gradient is in general again basipetal. This increase in susceptibility and the reappearance of the basipetal gradient in KCN is soon followed in my experiments by the beginning of death in the apical regions.

This apparent double reversal in the susceptibility gradient and the decrease followed by increase in susceptibility to KCN requires some consideration. This case illustrates the difficulties which immediately arise when susceptibility to one inhibiting agent or condition is used as the measure of the action of another. Until we know more concerning the changes preceding death in such a case as this, it is impossible to reach any definite conclusion concerning the meaning of the changes in susceptibility, but certain probabilities suggest themselves.

In the first place the decrease in susceptibility and the more or less complete reversal of the original gradient, the same changes which have been observed in various species and under various depressing or injurious conditions (Child, '16*e*, '17), undoubtedly indicate a differential depression or injury along the axis, the most susceptible, apical regions being so much more injured than less susceptible, more basal regions, that the original relations are often reversed. The point of interest is that changes of this sort brought about by high temperature, confinement, etc., appear in the altered susceptibility gradient to KCN. This apparently means that the action of these other conditions and that of KCN on the protoplasm are not exactly of the same sort, *i. e.*, not strictly additive, for if they were, we should expect the KCN simply to continue the action of the high temperature or confinement without making them visible as a reversed gradient and a decreased susceptibility.

As death approaches, however, at least in the confinement experiments, further changes apparently occur which differ from those of the earlier stages and these changes appear in increased susceptibility to KCN and a second reversal of the susceptibility gradient, a return to the normal basipetal condition. Under ordinary conditions such increase in susceptibility would be interpreted as associated with an increase in metabolic rate, greatest in the apical regions, but it is impossible to believe that in the later stages of confinement the cells approaching death undergo any such increase in metabolic activity as these changes would indicate. It must be rather that in the later stages of the decrease in metabolism which precedes death under these conditions changes occur which are essentially like those produced by KCN so that plants at this stage behave as if they had already been subjected to KCN, and the final killing by KCN is simply or largely additive to the changes which have already occurred. The action of the ordinary narcotics such as alcohol and ether, at least in its more advanced stages, and that of KCN are also additive and thoroughly narcotized organisms are more susceptible to KCN than normal organisms, though there can be no question as to metabolic condition in the two cases. Evidently changes in susceptibility produced by one agent or condition can be made evident only by some agent or condition whose effect is not strictly additive to the first.

It seems not improbable that the present case is merely a particular example of a general relation. In terms of the permeability theory, for example, the first effect of an inhibiting agent may be, or be associated with a decrease in permeability, and if this effect is proportional to the original susceptibility along the axis, it is not difficult to understand that one such agent might produce a differential decrease in permeability and so reverse the axial gradient in susceptibility to another. In the later stages of the action of an inhibiting agent, there may be on the other hand an increase in permeability resulting from changes in protoplasmic aggregation or other changes, and after this stage is reached the effect of one agent is essentially additive to that of another. This is not to be regarded as a hypothesis in the scientific sense to account for the facts, but rather as merely a suggestion to illustrate how susceptibility relations may be altered. This case also shows certain of the limitations and difficulties of the susceptibility method in demonstrating by means of one agent the effects of another. Some of these difficulties may be removed or lessened by working with the proper combinations of agents.

For present purposes, however, the changes during the earlier, not those of the later stages of confinement are important. These changes, consisting in decrease in susceptibility and more or less reversal of the general gradient, are the same as the changes which occur very generally, though more slowly, under laboratory conditions even in running water. There can be no doubt that the transference of the plants from natural to laboratory conditions (Child, '16*e*), like the earlier stages of confinement, brings about a greater or less degree of physiological depression which is greatest in the most sensitive parts of the plant, the apical regions, and which, if sufficient, obliterates or reverses the original axial gradient. In cases of confinement in the small flasks as described, death usually follows this change in two or three days at most, but where the change occurs less rapidly, as in a large flask, in open standing water or in aquaria with running water, the obliteration or reversal of the gradient in the apical region is followed after two or three days by separation of the cells of the apical region in which the obliteration or reversal of the gradient occurred. In short, after obliteration or reversal of the gradient together with decrease in metabolic activity, the axis falls apart into its individual cells or into groups of two or three cells. This process begins apically because this is the region where the change first occurs, but as time goes on, if the environment does not improve, or if the plant is unable to become acclimated as a whole, the change in the gradient extends farther and farther in the basipetal direction and the separation into cells follows it in the same direction. In plants kept in open crystallizing dishes in a liter of sea-water without change, cell separation usually begins in the apical regions after several days and progresses basipetally, until after ten days most axes are completely separated into cells or small cell groups. In running water also cell separation usually begins in the apical regions after several days and progresses basipetally, though more slowly than in standing water or in a closed flask. In this case, however, separation often ceases at some level of the axis and the cells basal to this level maintain their continuity. The apical beginning, the basipetal progress, and under the less extreme conditions the halting at one level or another of this process of cell-separation constitute still another demonstration of the existence of an axial gradient in which susceptibility to these conditions is greatest apically and decreases basipetally.

This process of cell-separation is a reaction of the living cell, and is not necessarily followed by death, although under conditions in which any considerable degree of separation occurs, a varying percentage of the cells, chiefly the cells of more apical regions, does die. In shallow crystallizing dishes with a liter of water fifty per cent. more or less of the cells may die, in more extreme conditions a higher percentage, up to one hundred per cent. in a closed flask, and under less extreme conditions a smaller percentage.

In all cases, however, where conditions are not too extreme a certain percentage of the cells may remain alive, and after a time resume growth and development. In short, these depressing conditions if not too extreme may be used as a method of obtaining isolated cells of *Griffithsia* for purposes of culture. This method is not new, for Tobler, '02, '04, has employed it for this purpose, but the fact that such cell-separation is associated with obliteration or reversal of the axial gradient is new. If, as I believe (Child, '15*b*), the axial gradient is the basis of physiological polarity, and at the same time the primary integrating factor in axiate individuation, the obliteration or reversal of the gradient must mean both obliteration or reversal of the original polarity and physiological disintegration of the individual, in other words, the elimination of the physiological order or pattern associated with and resulting from the existence of the gradient. With this physiological disintegration of the individual its component parts, the cells, become more or less completely physiologically isolated (Child, '15*b*). The fact that physiological disintegration of the individual is soon followed by physical separation of the cells is of great interest, as indicating the very direct dependence in this simple individual, of the gross morphological order upon the dynamic integrating factor.

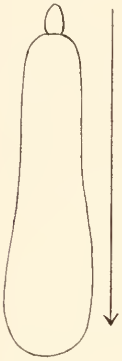
Moreover, if the gradient is the basis of physiological polarity, we may expect that the further growth and development of these isolated cells will be influenced in one way or another by the obliteration or reversal of the original gradient. If the original gradient is completely or to a large extent obliterated in a cell, a new gradient or gradients may be established by "chance" differentials in external conditions, or may be determined experimentally. If reversal of the original gradient persists for any considerable time, the order and axiation in the further development may be determined by this factor. In the following section the development of some of these isolated cells is described, and its relation to susceptibility gradients demonstrated.

THE DEVELOPMENT OF NEW AXES FROM ISOLATED CELLS IN RELATION TO THE GRADIENT.

One lot of *Griffithsia*, kept in running water for three days after collection, was then placed in a shallow crystallizing dish in one liter of water and left open to the air in diffuse daylight without change of water. After two days in standing water cell separation began apically, and after seven days had extended over the whole length of the axes. Most of the cells were single, but a few groups of two, three or even four cells were observed. About half of the cells were dead at this time, the death rate being highest among the cells from more apical regions. Almost every one of the apical cells and probably 75–80 per cent. of the first three or four subapical cells were dead. Taking the shape and size of the isolated cells as an index of their approximate level in the axis, it is evident that the proportion of cells killed is greatest in the most apical regions and decreases basally, at least, to the middle regions of the axis, below which the gradient is slight, even under normal conditions. In short, the susceptibility gradient of the axis is in evidence under these conditions as well as in more rapid killing.

After another week in standing water, examination showed new growth and development in 10–15 per cent. of the living cells. Figs. 2 and 3 show cells from the more basal levels with new apical cells at what were originally the basal ends. Susceptibility gradients were determined in both these cases, the progress of death being in the direction of the arrows, the new apical cell dying before the old, large cell and the intracellular gradient in the large cell being in the direction of the arrow in both cases. In many other similar cases similar gradients were found and still other cases were observed without determination of the susceptibility gradient.

Fig. 4 shows a cell from the middle region of an axis, in which a new apical cell has arisen at the original apical end. The susceptibility gradient, both general and intracellular, is indicated by the arrow which points in the direction in which death progresses. Fig. 5 is a case in which a group of three cells from a level near the apical end have remained connected. The only new growth in this case is a rhizoid from the basal end of the most basal cell of the group. Here the original susceptibility gradient persists in the three cells, as indicated by the arrow, while the rhizoid, like any other rhizoid, represents a new gradient in the opposite direction. The rhizoid, however, represents a lower level of susceptibility than other parts of the individual and is the last portion to die.



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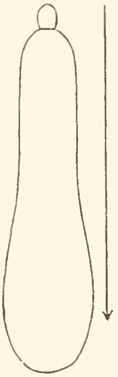

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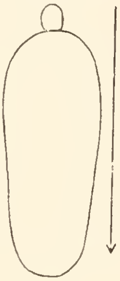

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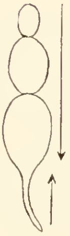

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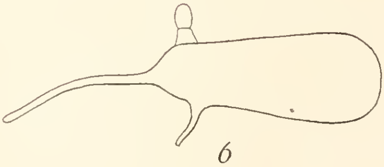

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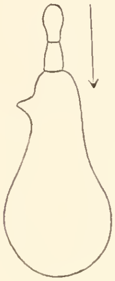
In Figs. 2 and 3 the original gradient has undergone reversal, in Figs. 4 and 5 it still persists. Cases of reversal, like Figs. 2 and 3, were in general more frequent in the material than cases of persistence. This is to be expected, since cell separation is associated with obliteration or reversal of the gradient. The original gradient may persist in cells or cell-groups in which the susceptibility is for any reason exceptionally low and the differential injury consequently less marked, and probably also in cases of partial acclimation (Child, '15*a*, Chap. III., '16*d*) after complete obliteration. It may be also that in some cells, where the original gradient had undergone reversal in connection with the basal growth, another reversal may accompany cell separation.

Fig. 6 shows a case in which a new polarity at right angles to the old appears in the basal region of a large cell. A new axis consisting of an apical and a subapical cell, has arisen from the lateral cell-surface near the basal end. The susceptibility gradient was not determined in this case, but there can be no doubt that the new axis represents a new gradient. The gradient in this case may perhaps have been directly determined by the differential action of an external factor, possibly concentration of oxygen or other substances necessary for metabolism. Cells lying on the bottom of the dish, or perhaps in contact with other cells, may often be subjected to such an external differential, and where the original gradient is to a large extent obliterated, a new gradient, and so a new axis may readily arise. Cases of this sort have been recorded for *Corymorpha*, among the lower animals (Child, '15*b*, pp. 142–146).

Unfortunately this culture of *Griffithsia* was made toward the end of the season, and my departure from Woods Hole prevented long continued observation. Through the kindness of Professor Osterhout, however, in turning over to me a culture of *Griffithsia* which had been in the laboratory some three months in very slowly running water, I was able to make some observations on more advanced stages of development of isolated cells. In this culture cell separation had occurred to a large extent, and many isolated cells showed considerable growth, although at the time of examination in many cases the new growths were dying or not in good condition. Figs. 7–10 show cases from this culture in which the susceptibility gradient was determined.

In Fig. 7 a new two-celled axis has arisen from the basal end of a cell, and the general susceptibility gradient, as indicated by the arrow, shows the usual relation to this axis, but the intracellular gradient in the large old cell is very slight.

Fig. 8 shows another case of a new axis consisting of three cells arising from the basal end of a large old cell and two new living rhizoids on the basal half and one dead rhizoid, indicated by dotted lines, on the original apical region of the old cell. The arrows indicate the gradients. Death begins in the apical cell of the new axis and continues down the large cell to its middle, while an opposed gradient exists at the opposite end of the large cell. It is of course impossible to determine the physiological history of this cell, but appearances suggest that the cell was one of those in which the gradient had been reversed and a



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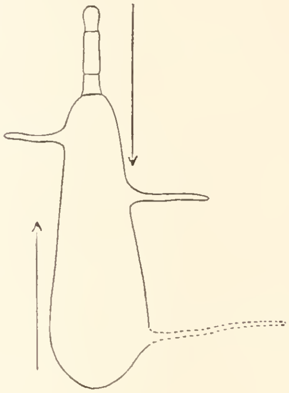

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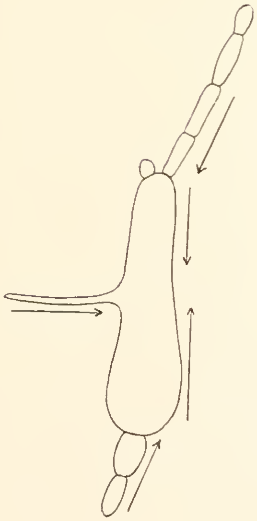

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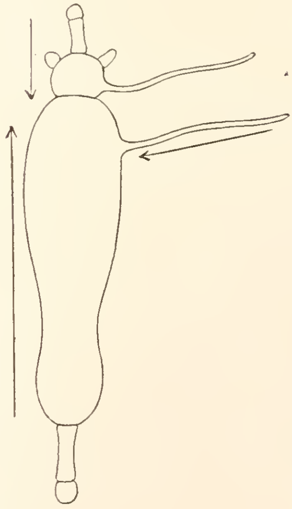

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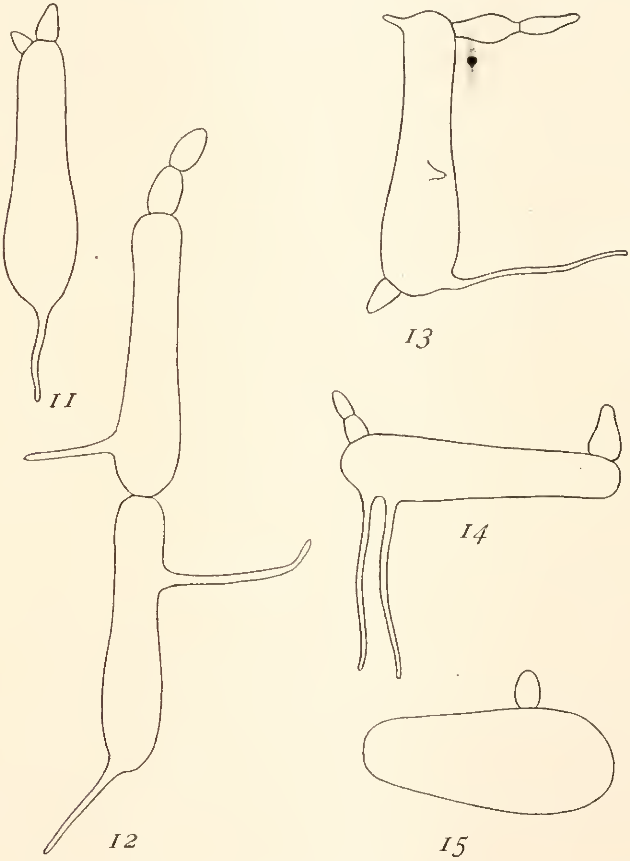
rhizoid had arisen from the apical region before isolation (see p. 215). After isolation the conditions determined a double gradient, the old rhizoid died and new rhizoids arose in relation to the new axis. The original apical end of the cell, although it represents the high end of a gradient, is not active enough to give rise to a new apical cell.

Fig. 9 shows a cell with two new axes, one of four cells, the other of one cell, at its original basal end, one new two-celled axis at its apical end and a rhizoid arising from its middle region. The gradients are indicated by the arrows. It will be observed that a double gradient exists in the old cell, the middle region, where the rhizoid arises, being the region of lowest susceptibility. The two axes at the basal end and also the rhizoid were found to be more susceptible than the new axis at the apical end of the old cell, and the old cell is least susceptible of all, and its middle region the least susceptible region.

Fig. 10 is another case with new axes at both ends of the old cell. At the original apical end is one three-celled axis with two one-celled branches and one rhizoid arising from its most basal cell. At the original basal end of the old cell is another axis of two cells and the old cell also bears a rhizoid near its apical end. In this case observation of the progress of death was interrupted, and the susceptibility gradients were observed only in part. Those observed are indicated by arrows. This case showed all indications of being in poor physiological condition. The small apical cells were very heavily pigmented and instead of being rounded were flattened basally, suggesting a low turgor. The protoplasm of the large cell had also undergone structural changes similar to those which precede death in KCN and other agents. The susceptibility of all the new axes, except the one rhizoid axis arising from the old cell, was somewhat lower than that of the old cell itself. In the absence of knowledge of earlier stages in the life history of this case it is impossible to determine the meaning of the susceptibility relations between new and old parts. In several other cases the new axes were found to be less susceptible than the old cell, and in every such case the new axis, or at least its apical cell was very deeply pigmented and flattened basally, and the protoplasm of the old cell had undergone more or less structural change. Apparently all such cases have ceased to grow and are, at least in large measure, quiescent, if not slowly dying. Their susceptibility is in general very low. Since this condition unquestionably results from the unfavorable environment, the new axes have been inhibited or injured to a much greater degree than the old cell because of the originally higher susceptibility associated with their higher metabolic rate. If this is the case the question at once arises, why cell separation has not again occurred. As a matter of fact cell separation was occasionally seen in the new axes, though not very frequently. Its infrequency, even where



the gradient is reversed, is probably due to the greater area between the cells which is a very general characteristic of the new parts developing under these conditions. The cell turgor is apparently less than under normal conditions, and the young cells therefore depart more or less from the spherical or ovoid form characteristic of young cells produced under normal conditions, and in consequence of this change the surfaces of contact become larger and separation occurs less readily.

Figs. 11–14 show other cases of development of isolated cells from Osterhout's culture, in which the susceptibility gradients were not determined. In Fig. 11 two new apical cells arise from the original basal end, and a rhizoid from the original apical end of the cell. In Fig. 12 a new axis of two cells has developed from the basal end of a group of two cells, and rhizoids appear at various levels on the old cells. In Fig. 13 the two ends of the old cell apparently develop independently of each other, each giving rise to a new axis and a rhizoid at approximately right angles to the original axis, and a third rhizoid appears in the middle region. Fig. 14 shows a somewhat similar case with a two-celled axis and two rhizoids developed from the apical region of the old cell and a one-celled axis from the basal region. It will be noted that in all these cases the new apical cells arise at or near one or both ends of the old cells. Among all the cells examined, both from Osterhout's and from my own culture, only one case was found (Fig. 15) in which a new apical cell arose from the middle region of the old cell. This case was returned to the culture after examination in the hope that further development would occur, but could not be found later. Numerous other cases of development occurred in the isolated cells of these two cultures, but those described give a fair idea of the variety of axial relations observed.

Discussion.

If the axial susceptibility gradient is in any degree an indication of the general metabolic relations along the axis, and if these metabolic relations are effective factors in determining the axial order or pattern, then the apical cell represents the region of highest metabolic rate, and in the vigorous axis under good environmental conditions a gradient of decreasing metabolic activity extends basally. The rhizoid itself is a similar gradient, and the internal condition which favors rhizoid-development is apparently a low metabolic rate, though external conditions may determine whether it actually develops or not. It is, in fact, like the stolon of the hydroid, *Tubularia*, a secondary gradient, originating as a local region of increased metabolic activity in consequence of partial physiological isolation (Child, '15*b*, pp. 91, 92), in which external factors probably, at least often, play a part (Child, '15*b*, pp. 132–134). The rhizoid, in short, represents a secondary, more or less subordinate gradient, originating at what may be called a certain metabolic level in the axis or between certain metabolic limits and probably developing in relation to certain external factors. This conception agrees well with the fact that the susceptibility of the rhizoid, even at its tip, is usually lower than that of the apical region of the main axis, while even at its base, its susceptibility is usually higher than that of the level from which it arose.

Rhizoid-formation is not necessarily limited to the extreme basal end of the axis, for as metabolic rate decreases with advancing age of the cells, other levels may attain a condition which permits rhizoids to develop, and rhizoids may therefore appear first at the base and later rhizoid-formation may progress acropetally along the physiologically older portions of the axis, as it actually does in various species of algæ (Tobler, '06) and to some extent and rather irregularly in *Griffithsia*. As regards their relations to the primary axial gradient the roots of the higher plants are probably similar to the rhizoids (Child, '15*b*, pp. 156–163), though their function is of course different.

Assuming the correctness of this conception of the simplest plant axis, we should expect to find that in cells isolated from a preëxisting axis new apical cells would arise from the region of the cell possessing the highest metabolic rate and rhizoids from the region of lowest rate, or at least from lower metabolic levels than the apical cell. So far as determined, the susceptibility gradients in the forms developed from isolated cells agree in general with this expectation. In every case from my own culture, where only the earlier stages of new axial development were present, the new apical cell arises from the region of highest susceptibility in the old cell. In the more advanced stages of development in the Osterhout culture most of the cases in which the susceptibility gradients were determined show the same relations, but some exceptions occur, apparently in cases which are in poor condition or gradually dying. Moreover, the fact that in these isolated cells new apical cells arise more frequently from the basal end or from both ends of the old cell than from the apical end alone is undoubtedly connected with the fact that cell separation is associated with obliteration or reversal of the original axial gradient in the cells. The greater frequency of development of new apical cells at or near one or both ends of the old cell than in the middle region is probably also an expression of gradient relations. Where the intracellular gradient was primarily basipetal and is completely reversed, the basal end of the cell becomes physiologically apical, and morphological development follows the new metabolic relations. Where the original gradient is largely obliterated, the two ends of the cell which were the regions of connection with other cells are doubtless more affected than other parts of the cell by the change in conditions accompanying the separation and undergo a certain amount of regression and rejuvenescence, and so attain a higher metabolic rate (Child, '15*a*, Chap. X.). It is also possible that in some cells from the more basal regions, where secondary basal elongation is occurring, and where two opposed intracellular gradients are often present (see p. 215) before separation, the obliteration of these gradients is not complete at the time of separation, and the higher rate at the two ends is still further increased by separation, so that both ends become physiologically apical. And finally, where the gradient is reversed in the normal plant, as sometimes in the more basal cells, it may be again reversed, obliterated or almost obliterated at the time of separation with correspondingly different results in later development.

While it is impossible to follow the changes in the gradient in a particular cell and to correlate them with the later development, the high degree of constancy in the relation between the intracellular gradients and the localization of the new axes leaves little room for doubt concerning the fundamental character of the relation.

The cases where the new axes arise from the lateral surface rather than the end of the cell, as in Figs. 6, 13, 14, or from the middle region, as in Fig. 15, cannot be definitely accounted for, but are doubtless connected with special external conditions, perhaps the position of the cell with respect to light, oxygen supply or other external factors, or perhaps with local metabolic differences in the cell produced in some other way. I believe, however, that if we could analyze these cases, we should find that they, like the others, are an expression of the existing gradient relations, however these may have been determined (cf. Tobler, '06).

The localization of the rhizoids also agrees in general with expectation as far as the data go. In general they arise from a relatively low metabolic level on the cells, but this level may be in the middle region (Fig. 9) or at or near one end (Figs. 5, 6, 11), or rhizoids may appear progressively over a considerable length of the cell or cells (Figs. 8 and 12).

As regards the facts of morphological development, these experiments add nothing of fundamental importance to those of Tobler ('02, '04, '06), but the determination of the relations between the susceptibility gradients and cell separation and the localization of new axes serves to throw some light on the physiological conditions concerned in this development and on the problems of physiological polarity and physiological integration or individuation.

These experiments suggest further possibilities of more exact control of the localization of new axes on isolated cells, such as the determination of the influence of the direct subjection of single cells to the differential action of external factors on the localization of new axes.

Summary.

1. The original axial susceptibility gradient of the alga *Griffithsia* is more or less completely obliterated or reversed after one or two days of confinement in a small volume of water, and the larger the volume of living plant protoplasm introduced, the more rapid the changes.

2. Where the conditions of confinement are extreme, obliteration or reversal of the gradient is followed in one or two days by death with a basipetal gradient, but under less extreme conditions the alteration of the gradient is followed by the separation of the axis into individual cells or occasionally small cell groups, the separation beginning apically and progressing basipetally.

3. Obliteration or reversal of the original axial gradient, followed by cell separation occurs, though more slowly, when the plants are kept in open standing water and to a greater or less degree, but still more slowly, in slowly running water in diffuse daylight.

4. A variable percentage of the isolated cells dies, the death rate being in general highest in apical cells and decreasing with the level of the cell in the axis, at least to the middle levels, below which there is often little difference. The cells which do not die may undergo a new development, giving rise to new apical cells and so to new axes.

5. Determinations of the susceptibility gradients in these reproductive developments from isolated cells show that the new apical cell arises from the region of highest susceptibility in the old cell. This region is more commonly basal than apical, because the gradient is usually reversed at the time of cell separation, but both ends of the cell may give rise to new apical cells where double opposed gradients exist. Rhizoids, on the other hand, arise from low levels in the axial gradient.

6. These experiments show that the original polarity of the cells of *Griffithsia* can be completely obliterated or reversed, and that the morphological development of new axes is an expression of gradient relations present in the cell at the time.

7. The facts support the conclusion that a gradient in metabolic rate, protoplasmic condition, or whatever we prefer to call it, of which the susceptibility gradient is within certain limits an indicator, constitutes physiological polarity in protoplasm, and that such a gradient is not an inherent property of protoplasm, but may be determined and altered by external factors.

HULL ZOÖLOGICAL LABORATORY, UNIVERSITY OF CHICAGO,
January, 1917.

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THE EFFECT OF ETHER AND CHLOROFORM ON CERTAIN FISHES.[1]

HAROLD D. CLAYBERG.

PAGE.
I. Introduction 234
II. Reactions 235
  1. Material and Methods 235
  2. Reactions and Standardization 236
  3. Chloroform 238
  4. Ether 238
III. Resistance 239
IV. Comparison of Ether and Chloroform Killing Curves 243
V. Poisoning Symptoms 245
VI. Summary of Conclusions 247
VII. Bibliography 247

I. INTRODUCTION.

Various drugs which cannot be standardized chemically must be standardized biologically before being used for medical purposes. The time required to kill an animal (for example, a goldfish) has been used for this purpose (see particularly Pittenger and Vanderkleed, 1915). The discussion about digitalis has been particularly keen. The effect of a large series of coal tar derivatives upon various wild fish has been studied by Shelford ('17) in connection with stream pollution work. He determined their relative toxicity by killing specimens of the orange-spotted sunfish. The work described below was undertaken with the two common drugs, ether and chloroform. Their effect on the orange-spotted sunfish and on certain other wild species was studied in the hope that it might serve to relate the coal tar results with those of the investigators interested in the standardization of drugs with domestic goldfish.

It is the purpose of this paper to point out that there seem to be three possible ways of standardizing a drug with fish.

1. Type of behavior in a gradient between pure water and water plus the drug being tested might be used. To furnish

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[1] Contribution from the Zoölogical Laboratories, University of Illinois, No. 90

definite standards a distinct form of behavior should appear or disappear at a definite concentration.

2. Time till definite symptoms of sickness appear might be used. Such a condition for serial concentrations is shown to the left in chart II.

3. Killing time is the only standard thus far applied.

In connection with killing it is our purpose to point out that the time-concentration curve is probably an hyperbola which, if fully demonstrated for a few substances, will render the investigation of drugs in general much easier.

II. Reactions.

1. *Materials and Methods.*—The reaction experiments were performed in the Shelford gradient tank. This is a tank 122 cm. long by 15 cm. wide by 13 cm. deep. Water is allowed to flow in at each end at a definite rate. It enters the tank through two tees, the cross bars of which are perforated with small holes so as to distribute the inflow throughout the width of the tank. It flows out at the middle at both top and bottom so the two kinds of water meet at the center. In these experiments normal water was run in at the left end at a rate of about 450 c.c. per min. and normal water plus ether or chloroform was run in at the right end at an equal rate. The drug solution was made up in an 18-liter bottle, placed above the apparatus, and siphoned out. Siphon and bottle were closed on account of the volatility of the poisons used. To be certain that the concentration under observation remained constant, the flows were tested occasionally throughout the experiment; hence the concentrations quoted are presumably accurate. The outflow at the center did not prevent the mixing of the two kinds of water and thus the middle section equal to one half or third of the tank, was a gradient between the normal water and water plus ether or chloroform. Accordingly as a fish moved from left to right in the tank it encountered a gradually increasing concentration of the poison. We found no evidence that fish react to the slight current through the tank. Since each half of the tank held about 9 liters, it required 21 minutes to fill it or to replace all the water in one of the halves.

Two 8 candlepower electric lights were fixed above the center of the two halves, *i. e.*, above a point midway between the screen partition in front of the introducer tees and the center drain. The lights were 15–20 cm. above the surface of the water which was 13 cm. deep. The tank and lights were enclosed under a black hood. The experiments were observed through openings in the hood above the lights or through the glass side late at night. Water differing as little as possible from that in which the fish usually live was used for control readings. Controls were observed and the conditions in the two ends of these were the same either because the water introduced at the two ends was alike or because no water was run into either end (standing water). Behavior of both bullhead and sucker in tank controls was of two types identified as *quiescence* and *circuiting.* The latter refers to swimming around the tank and involves both back and forth movement and crosswise movement. The crosswise movement, while ignored in the charts, should be understood to occur, especially in the case of controls. In controls quiescence predominated after a short period of circuiting (see Chart I., Bullhead Control).

In experiments of this kind the reactions of the fish are commonly graphed, that is the distance between two lines on a sheet of paper (see chart I.) is used to represent the length of the tank. Cross movements of the fish are ignored in the graphing. Vertical distance is used to represent time according to scale. Thus in chart I. control 3 the bullhead went the entire length of the tank twice during the first one minute and 20 seconds.

For the reaction experiments only suckers (*Catostomus commersonii*) and bullheads (*Ameiurus nebulosus*) were available, but their behavior is not materially different from that of the standard sunfish (*Lepomis humilis*). More fish were not obtainable on account of the swollen condition of the streams, hence the small number of experiments done.

2. *Reaction and Standardization.*—Standardization through reaction in gradients would be dependent upon (*a*) Definiteness of some change in reaction occurring (in case of the fish); (*b*) accuracy of the change with reference to concentration; (*c*) speed of standardization; (*d*) reasonably low cost of the process.

CHART I.

Graphs of Reaction of Fish to Chloroform and Ether.

(See Table I.)

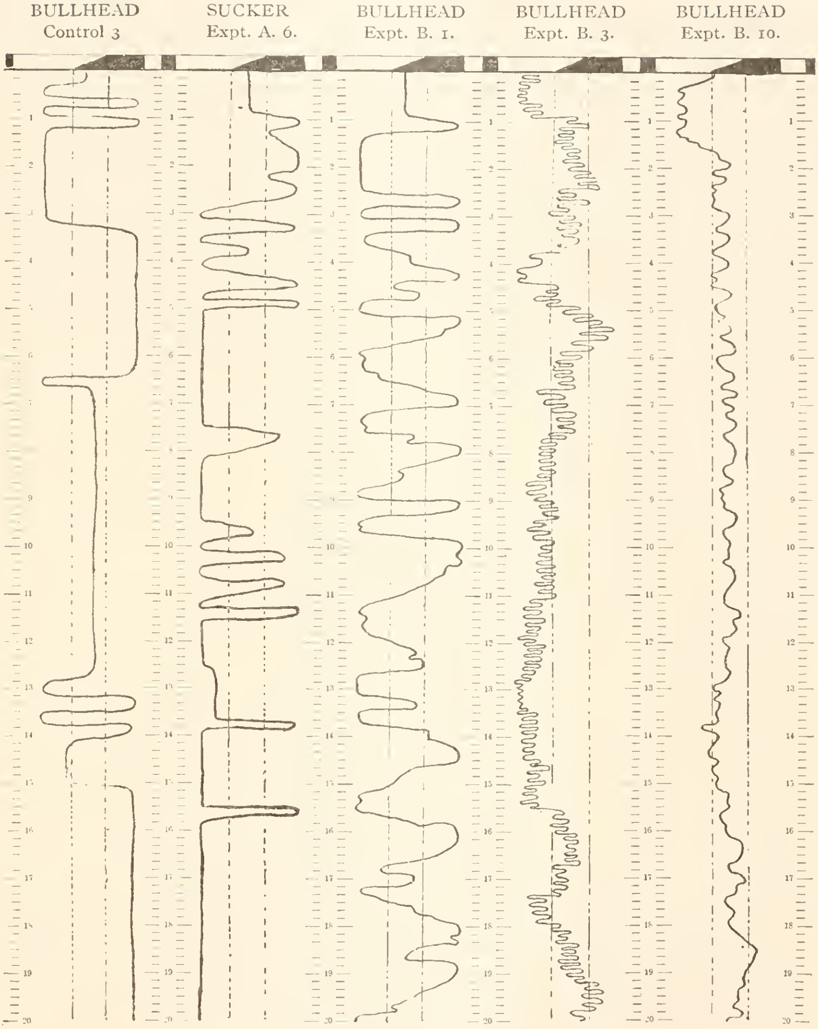


CHART I. Reaction graphs. The drug was introduced at the right; its concentration is indicated by the larger black area in the heading. These graphs represent parts of experiments only and are meant to show, primarily, the type of action of the fish to certain poison concentrations in the gradient tank. To the extreme left is shown a control experiment on a bullhead and it should be compared with the three to the right (of series B) in order to bring out the distinctive types of behavior involved. Not considering the sucker experiment and taking only those shown on the bullhead it is plain that an intergradation of action can be traced from the one on the extreme left to the one of the extreme right.

The reaction of the sucker to chloroform is shown in the second graph to the left and should be compared with the reaction of the bullhead to ether as shown in Expt. B. 1 for the concentrations are approximately isotoxic; the bullhead showing notches which are never present in the charts of suckers.

The following studies were carried out with the idea that a reaction might prove more definite, and perhaps less variable than time of death.

3. *Chloroform.*—Bullheads and suckers were used in a series of experiments which began with low concentrations which were increased in the successive trials. Both species showed considerable individual variation and in general failed to react to most concentrations. Both species showed some positive reactions in the case of .14 c.c. per liter (213.8 mg.) and a few negative reactions occurred in somewhat higher concentrations. There appeared to be no definite relation between reaction and fish size. The general failure to give definite positive or negative reactions may be due to a paralysis of the sensory endings with which fishes are known to recognize substances in solution in water.

4. *Ether.*—Bullheads alone were used, but a complete series of ten experiments were run in which concentration ranged from .267–4.573 c.c. per liter (192.24–3292.56 mg.). These solutions were all distinctly non-fatal because no symptoms of even partial loss of equilibrium were observed. Although some experiments ran for three hours, the fish exhibited no true positive or negative reaction when any experiment as a whole was considered. If, however, a small piece of one were carefully chosen an impression of reaction in either direction could be obtained from any experiment. The piece chosen from experiment B. 3 on chart I. was chosen for another purpose but it appears to show negative reaction. This is not the case as the whole experiment rather points to indefinite reaction.

While tank end preference was nil, the general behavior of the fish underwent definite change twice during the gradual increase in strengths of the poison used. Chart I. contains critical experiments of which repeated mention will be made through the rest of the reaction discussion. Experiment B. 1 was the first of the series and shows that circuiting predominated; but occasional little notches occur in the curve that are absent in the sucker's curve (exp. A. 6) and which are very suggestive of the behavior of the bullhead to higher concentrations. Thus experiment B. 1, having a concentration of .267 c.c. per liter (192.2 mg.), fixes a definite sort of behavior for comparison with later experiments.

After 100 minutes in this .267, or after 13 minutes in .403 (290.1 mg.) a very distinct third type of reaction appeared. This might very appropriately be named the *nosing reaction.* It was characterized by incessant restlessness and continual change of direction; the fish nosed back and forth in a peculiar uneasy manner, turning around 180° every 2–8 seconds, holding its head always close against the glass side of the tank, and keeping this up for hours. Experiment B. 3 shows how remarkably this reaction differed from the others. In concentration 4.573 (3,292.5 mg.) this nosing reaction was entirely eliminated, and a still different type of reaction appeared (see exp. B. 10).

This reaction might well be designated as *intermediate,* since it seemed to be a compromise between the circuiting of exp. B. 1 and the nosing reaction of higher concentrations, in that it exhibited the slow turning of the former combined with the narrow range of the latter. The range of the nosing reaction in relation to concentration is shown in Table I. It seems that such an apparently precise reaction ought to be found with fish or other aquatic animals in the case of drugs needing standardization. Comparison of Table I. with parts of three experiments shown to the right in chart I. will make clear the significance of the results attained.

TABLE I.

SHOWING RELATION BETWEEN ETHER CONCENTRATION AND TIME TILL INCEPTION OF THE NOSING REACTION IS OBSERVED.

Series B. Exp. Number.	Poison Entry Rate in Cu Mm./Sec.	Poison Concentration at Right End of Tank.		Time till Nosing Reaction Started, Minutes.	Duration of Expt., Minutes.
		C.c. per Liter.	Mg. per Liter.		
1	2	.2667	192.0	100	128
3	3	.6667	480.0	30	182
5	10	1.3333	960.0	10	86
9	30	3.7855	2725.6	0	97
10	35	4.5733	3292.6	*	72

* No nosing reaction whatever occurred throughout this experiment.

III. RESISTANCE.

1. *Materials and Methods.*—Killing of fish was carried on in closed bottles in which poison solutions of known concentrations were made up. As a rule 4-liter bottles were used, although

some of 2- and 5-liter were also utilized. The fish was placed in the solution after being measured and weighed; and the time till its behavior became definitely abnormal and the time till death were carefully noted.

2. *Results with the Minnow* (*Pimephales*).—Preliminary work was undertaken looking towards a comparison of the closed bottle method of killing (used in all drug standardization work) with that of running poison of known concentration at definite rate through a bottle. The results indicated that the two methods had like effect for the same poison concentrations· Further, the results showed that the hour killing concentration for the minnow was between 2.2 and 2.3 c.c. per liter for ether (1,584–1,586 mg.). Weight used varied from 1.5–4 grams.

3. *Ether: The Sunfish.*—The standard used by Shelford for the measurement of toxicity of poisonous polluting substances is the concentration of any poison sufficient to kill a 4–5 gram *Lepomis humilis* in approximately 60 minutes. The hour killing concentration for ether was found to be between 3.685 and 3.690 c.c. per liter (2,653.2–2,656.8 mg.). Apparatus at hand permitted no closer determination. The number of experiments run was sufficient to plot the death curve shown in chart II. The base line or X-axis was used for minutes until the fish became abnormal or dead. The Y-axis was used for the concentrations employed. Points for all experiments were plotted and these were connected to form a curve. This curve showed a certain width due to variation in time of death throughout, which has been brought out by stippling.

The data were now separated into three classes to enquire into the effect of fish weight on death time. Since not enough fish of strictly standard size could be obtained, fish of size between 2 and 25 grams were used. Those obviously outside the weight from external appearance were used to determine the critical concentration roughly and those nearest the proper standard were used last. These were now placed in three classes: class 1, 2–9 grams; class 2, 10–19 grams; class 3, 20–25 grams. These were plotted in three curves on chart II. Those of classes 1 and 2 were complete enough to show the following facts.

At about 4.8 c.c. per liter (3,456 mg.) the fish of the two classes

CHART II.

Death Curve of Lepomis Humilis in Ether.

$((C_2H_5)_2O.)$

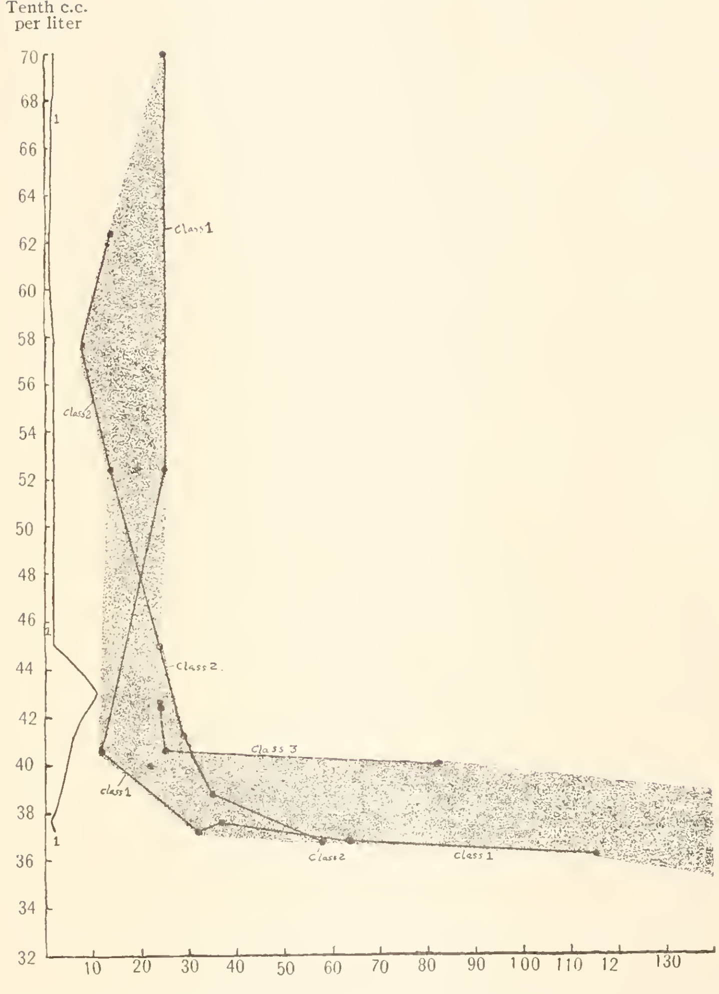


CHART II. (Ether death curve.) The curve to the left is the "abnormal curve" and marks the inception of symptoms such as loss of balance. The obvious fall in toxicity below 420 cu. mm. per liter is shown by the stippled death curve turning off to the right. Class 1 was of fish 2–9 grams in weight; class 2 was of fish 10–19 grams in weight; class 3 of fish 20–25 grams in weight. The significance and curve directions are discussed in the text. It is very likely there would have been much intercrossing if many experiments had been run and ten or more classes had been separated. It may be that difference of physiological condition of different batches of fish (as chance allots in the purchase of fish for standardizing) may give results with the same concentration of the same drug as far apart as the different weights of fish here have done. In the case of the fish used here all were caught in the same place, and kept in constant environment in the same tank until used.

The upper and lower boundaries of the curve at the lower right hand edge of the cart were determined by experiments not shown in the graph. I am indebted to Miss Dorothy Wing, of the University of Chicago, for aid in the preparation of this chart.

died in the same time. Above that concentration the toxicity of the poison increased for the 10–20 gram fish and decreased for the 2–9 gram fish up to about 5.3 c.c. per liter (3,816 mg.), beyond which it remained constant. Below about 4.8 c.c. per liter, on the contrary, the toxicity of ether increased in the case of class 1, and decreased in class 2 to a maximal difference at about 4.1 c.c. per liter (2,952 mg.) below which the two converged until they met at 3.7 (2,664). Their crossing point is at the hour death concentration. If, at certain points, the death curves of different weights of fish are found to cross, in standardizing aconite, for example, it would be well to take these points as the standard and disregard the hour standard or any other previously set.

The third class furnished complete data only between 4 and 4.35 c.c. per liter (2,880 to 3,132 mg.). At the upper concentrations the toxicity is intermediate between classes 1 and 2. But below 4.1 c.c. per liter (2,952 mg.) the toxicity falls away far more rapidly than for the others. It is very likely that concentrations below 3.9 (2,808 mg.) will cease to have toxic effect of any sort on class 3. Since the critical concentration is well below this point the significance of weight of the fish used is seen to be of the utmost importance. It is regrettable that no large fish were available to test out the end of the class 3 curve. Very small fish below 2 grams are, as might be expected, killed in much less time than classes 1 or 2. Thus the resistance of the fish is seen to be consistently different for different weights and also of considerable complexity.

The crossing of the curves of classes 1 and 2 renders both classes of data available in the determination of the hour death point. The above results point out that, in standardization of drugs by fish, the different sizes may well prove to differ consistently and considerably. For this reason it would seem that the weight problem needs careful analysis and extensive experimentation to determine the proper weight limits to be allowed for results of the accuracy demanded.

Examination of the fish led to the graphing on the same sheet (see chart II.) of the time it took the fish in any concentration to become definitely abnormal (loss of balance, etc.). Above 4.5

c.c. per liter (3,240 mg.) the time was 1–2 minutes but below this concentration there occurred either a sudden decrease in initial toxicity or an equally strong increase in the resistance of the fish. This culminated at 4.3 c.c. (3,096 mg.) per liter in an "abnormal" time of 11 minutes, and from there, with further *decrease* in strength of solution the initial toxicity *increased* to the norm (1–2 min.) at a concentration of 3.7 c.c. per liter (2,664 mg.).

If a relation such as this be found for fish in standardizing some drug, it will be both quicker and cheaper to use it instead of death. Decreased initial toxicity with decrease in strength and close approach to hour killing concentration would be in no wise remarkable if it did not increase again to the norm as the concentration fell to the hour killing concentration as determined. But enough fish were used to render this inexplicable phenomenon reasonably certain.

4. *Chloroform: the Sunfish.*—The series run here were also plotted as explained for ether. See chart III. The hour death concentration lies between about .07 and .1 c.c. per liter (106.89 and 152.7 mg.) and much variation is exhibited between these points. The rest of the curve shows no such variation but it may be that this was due to the small number of experiments used to determine them. It is equally likely that only certain limited regions exhibit variability. Should this be the case in a drug needing standardization the variable areas should be located and no standard set anywhere within one of them. Continuous variation throughout a curve would, if wide enough, forbid the use, for accurate standardization work, of the experimental animal used in making the curve.

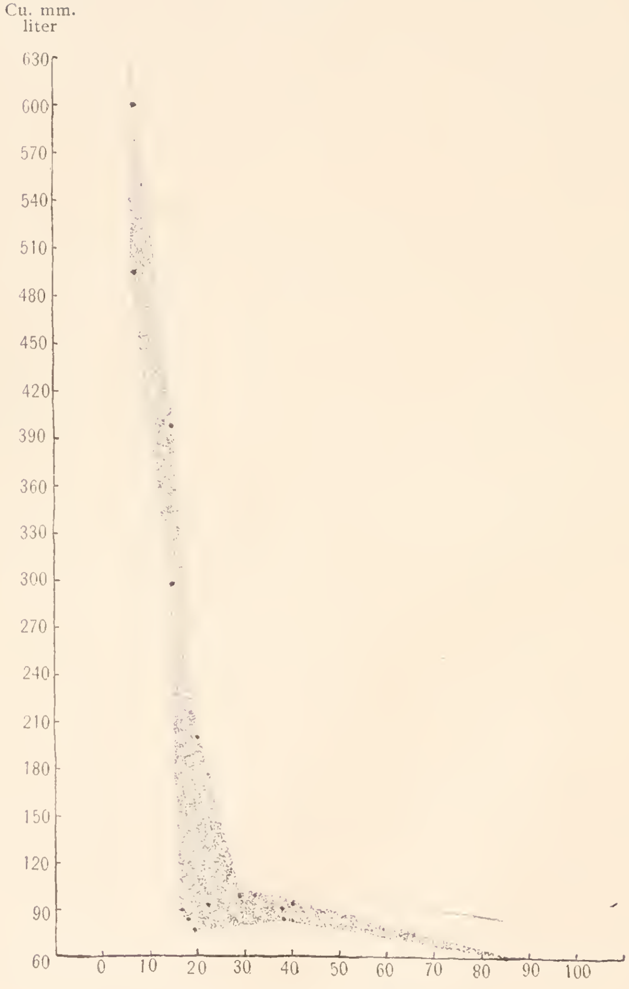
IV. COMPARISON OF ETHER AND CHLOROFORM KILLING CURVES.

It has been stated that these curves are hyperbolæ or very close to them. It should be further noted that the maximum death variability seems to coincide with that part of the curve showing greatest curvature. The laws governing hyperbolæ will probably be found to be of great use in fish standardization since they seem to die according to one. Twenty-four experi-

CHART III.

Death Curve of Lepomis Humilis in Chloroform.

($CHCl_3$.)



Time Until Death — Minutes.

CHART III. (Chloroform death curve.) Each point represents a separate experimental result. The poison is obviously very toxic as a concentration of less than .09 c.c. per liter kills in an hour. There is a sudden change in the curve between the concentrations of 100 and 200 cu. mm. per liter as if a new factor suddenly entered into the determination of the curve below the limits indicated. Only four of the fifteen fish used were above 6.2 grams and these were all used to determine the curve above 190 c. mm./liter. Hence no classes by weight could be separated.

ments were used to determine the ether curve and 15 for that of chloroform. This does not seem to be sufficient data to establish a law although it appears obvious that there may be one here. The upper limb of the chloroform curve is nearly a straight line and in it $c \times t = k$, about 4,200, c being concentration in cu. mm. per liter and t being time until death. It is probable that time, weight of fish, and concentration of poison used, will prove the determining factors for relative toxicity.

The similarity of the chloroform and ether curves suggest that after the location of a toxic zero, Krogh's ('14) Law may be found to govern under certain limitations. Should a law be here discovered and prove to hold closely enough, it is easily seen that it will prove of very great value in the standardization of poisons by means of fish.

V. Poisoning Symptoms.

1. *Ether.*—No particular irritability or spasmodic movements were noted. The fish at first acted normal, then swam vertically with head up, lastly quieted down and lay on its back or side, very usually at the surface of the water.

2. *Chloroform.*—For anæsthesia with either chloroform or ether Cushny[1] notes three stages: (1) Imperfect consciousness, (2) excitement, (3) anæsthesia. In fish, although the first stage is almost impossible to distinguish from the normal, the others may be definitely noted. The second stage is characterized through all concentrations used by a variable period of violent muscular contraction, which throws the fish wildly in all directions so that it bumps its nose strongly on the glass each time. Each bump is followed by a motionless pause of one half to one second. The third stage, coma, is reached in a time increasing as the concentration decreases. The fish lies on its side, or rarely on its back, at the bottom of the jar and the opercular movement is regular at first, then intermittent, and finally ceases. While the fish respires regularly it may recover if held under a stream of running water.

3. *Comparison of Ether and Chloroform.*—(*a*) In ether the stage of excitement is short or omitted while in chloroform it is

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[1] Pharmacology, p. 151.

obvious and protracted. (*b*) In the sunfish ether produces good anæsthesia allowing easy recovery, while chloroform poisons rather than anæsthetizes. (*c*) Honigman[1] states that with man chloroform is approximately three times as strong as ether (by weight). With the sunfish it is about 44 by volume or 20.4 by weight. (*d*) From previous statements it might be well to add that, if death remain the biologic change used for standardization, in certain cases it may be that other killing times may prove more accurate than the 60-minute standard.

4. *Comparison of Ether and Chloroform with Coal Tar Derivatives.*—Shelford ('17) has investigated the effect of various coal tar derivatives on standard fish. In comparing these poisons with the drugs previously discussed in this paper the following method was used. The poisons were put up in concentrations twice as strong as would be necessary to kill in an hour with the object of killing the fish in approximately 15 minutes. A specimen of golden shiner (*Abramis chrysoleuca*) was put into a 2-liter bottle of each poison solution and when it was dead a second was inserted. The actions of the fish were noted carefully. In all cases the opercular movement was greatly increased in amount and rate at the beginning of stage 2 (excitement) and later weakened and decreased. The action of these coal-tar derivatives as irritants and poisons was like that of chloroform but distinctly different from that of ether. A large amount of blood appeared to collect at the ventral side of the body toward the end of stage 2. This was notably so in quinoline, less so for aniline and toluene, and observable in the others. No such phenomenon was observed in the parallel chloroform and ether bottles.

TABLE II.

Poison Used.	Concentration in Cu Mm./Liter.	Time Different Fish Were Killed In.	Relative Irritability.	Opercular Rate Range Observed During Experiment
Toluene........	150	15, 23	Highest at first.	60– 88
Quinoline......	100	12, 20	Longest.	27–144
Aniline........	2,000	8, 9, 10	Very strong.	30– 60
Orthocresol.....	100	47	Medium.	70–240
Chloroform.....	600	7, 18	Strong.	22–180
Ether..........	4,250	18, 20	Very little.	90–102

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[1] Cushny, *op. cit.*, p. 163.

VI. Summary and Conclusions.

1. The behavior of bullhead and sucker in gradient tank to chloroform was usually indefinite and followed no rule.

2. Bullheads exhibit a peculiar nosing reaction in ether concentration (in gradient tank) between .3 and .4 c.c. per liter but not above or below these limits (except as noted). Such definite and remarkable changes in behavior may be determining factors in use of fish for standardization of poisons.

3. Concentrations of about .07 c.c. (106.89 mg.) per liter chloroform or of 3.69 c.c. (2,656.8 mg.) ether can kill standard sunfish in an hour.

4. In ether during third stage of anæsthesia the fish usually lies belly up at the surface while in chloroform it tends to lie on its side at the bottom of the bottle.

5. Toxicity ratio between chloroform and ether is 3 : 1 (by wt.) for man and 20 : 1 (by wt.) for the sunfish. Such constants may be of critical value in comparing the relative usefulness of different animals for standardization uses. It may not be the animal nearest man as regards toxicity ratio which is most valuable for standardization. For one where the toxicity ratio is far greater, and hence an animal far more susceptible, may be most accurate in perceiving and reacting to, or in dying from, the poison demanding measurement. Nearness or likeness to man is of little account and precision of reaction or death the paramount issue.

6. Ether anæsthetizes the sunfish while chloroform acts more as a poison, being, in a certain way, intermediate between ether and the violently irritating coal-tar derivatives studied above and in Shelford's work.

I take this opportunity to express my thanks to Professor Victor Shelford, of the University of Illinois, for the courteous help and kindly encouragement which made this paper possible.

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THE HISTORY OF THE CHROMOSOMAL VESICLES IN FUNDULUS AND THE THEORY OF GENETIC CONTINUITY OF CHROMOSOMES.

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At the Columbus meeting of the American Society of Zoölogists in 1915, the writer reported the occurrence of chromosomal vesicles which could be recognized as persistent entities even in the resting stage. Since that time it has been possible to trace the history of these vesicles from the metaphase condition of the chromosomes through the anaphase, telophases and the various stages of interkinesis back to the prophase stage where the new chromosomes are fully formed as new bodies which are as distinctly chromosomal in character as the more familiar ones of the metaphase. This history can now be given with but few gaps; and these are of comparatively little importance. The bearing of these facts on the hypothesis of genetic continuity of the chromosomes is direct and very important.

The presence of persistent chromosomal vesicles was first noted in the study of the eggs of *Fundulus heteroclitus* which had been fertilized with the sperm of *Ctenolabrus* (*Tautogolabrus*) *adspersus*, and the history was first made out in a general way upon those cleavage nuclei. Subsequently the observations were extended to the *Fundulus* eggs fertilized normally by the sperm of the same species, and the gaps in the series filled in from this form. So far as could be determined there is no essential morphological distinction in the figures presented by the normal and the hybrid nuclei. The same account applies to both, with the exception of certain points to be noted later, which do not apply to the general facts and line of argument here given.

The first observations were made while working on the hybrid eggs to determine the effect of radiation upon the development, particularly with the reference to the chromosomes which differ in form and which can be recognized as distinct in the hybrid eggs as was shown by Miss Morris following the lines of the classical work of Moenkhaus.

In 1904 Moenkhaus's well-known paper appeared announcing the independence of two chromosome groups on the spindles of dividing eggs of *Fundulus* and *Menidia* hybrids, and the recognition, based on morphological evidence, of the two groups as from the male and female parents respectively. Conklin (1901) had already recognized the male and female halves of *Crepidula* cleavage nuclei; Ruckert, (1895) and Häcker (1895, 1902) had seen the double nuclei and bilateral distribution of chromatin in *Cyclops;* and Herla (1893) and Zoja (1895) had traced the independent maternal and paternal chromosomes of *Ascaris* eggs to the twelve-cell stage. Moenkhaus, after identifying in the normal eggs the chromosomes of *Fundulus* as straight rods and those of *Menidia* as shorter curved rods, easily distinguished the male and female contributions to the cleavage nucleus of the hybrid eggs.

Since then a great deal of work has been done on the hybridization of many animals, and some further studies have been made on the crossing of different species of fish. In this group of animals, however, cytological observations have not been much extended. Yet G. and P. Hertwig figure a double spindle showing the portions derived from the two parents, and Miss Morris has corroborated Moenkhaus's principal findings in her crosses of *Fundulus* and *Ctenolabrus*. In the latter case, the *Fundulus* chromosomes, which are small straight rods, as Moenkhaus found them, differ clearly from those of *Ctenolabrus*, which, according to Miss Morris, are small and round.

At Woods Hole[1] in the summer of 1914 I repeated these experiments of Miss Morris on crossing the eggs of *Fundulus* with *Ctenolabrus* sperm. Since the reciprocal cross is not easily made, and since the fact as to whether the cross is possible was not under investigation, my experiments were limited to crossing in the one direction. It had occurred to me that some light might be thrown on the question of the effects of radioactivity on chromatin by treating variously with X-rays the eggs and sperm in these crosses where the chromosomes in the two differ. Experiments in four series with controls were therefore set up, and were treated with long and short exposure to X-rays as follows (adopting the series designations used by the Hertwigs in their radium experiments). Radiation treatment is indicated by *r*; untreated, or normal eggs and sperm are represented by *n*.

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[1] I am greatly indebted to the Director of the Marine Biological Laboratory for his kind assistance in providing me with the facilities of the laboratory.

Controls. *nF*♀ × *nC*♂ unradiated.
Series A. *nF*♀ × *nC*♂ radiated after fertilization.
Series B. *nF*♀ × *rC*♂.
Series C. *rF*♀ × *nC*♂.
Series D.. *rF*♀ × *rC*♂.

In general my observations corroborate those of Miss Morris as to the regular course of the events in the hybridized eggs of *Fundulus* and *Ctenolabrus*, and I find very little divergence in the behavior of the definitive chromosomes here from that described by Moenkhaus for *Fundulus* and *Menidia* crosses.

In general also, my results are in line with those of the Hertwigs in their radium experiments as to the injurious effects of the radiation upon the chromatin, shown, for example, in the failure of the egg nucleus in the series C, when strongly radiated, to take part in the development of the egg, as can be determined by the kind of chromosomes present on the spindle. Similarly, strongly radiated sperm chromatin in series B is killed and takes no part in development.

In the course of the investigation upon the radiated eggs a set of facts was discovered which bear directly upon the ever recurring question of chromosomal continuity, and which seemed to the writer, worth following out. It is the purpose of this paper to set forth those findings, and to show their bearing upon the important problem of cell structure, and to give at this time the results of the radiation experiments only very briefly, leaving them to be presented elsewhere.

During the early stages of the investigation the material studied was chiefly that which had been radiated, and most of the drawings given are from those slides. Subsequently, however, eggs of *Fundulus* which were unradiated have been sectioned, and studied from the same point of view and the conditions ascertained to be exactly the same as far as the formation of the chromosomal vesicles and their history is concerned. I wish to emphasize that neither the fact of the hybridization in the *Fundulus* ♀ × *Ctenolabrus* ♂ crosses nor the exposure to the X-radiation is responsible for the conditions here figured, for a full set of figures from normal *Fundulus* eggs in addition to those here presented could easily have been made and exactly the same conditions depicted, had the differences been thought to warrant the extra effort and time. To neither of these two experimental factors can the conditions here described be attributed.

It is quite possible and indeed the writer inclines to this opinion, that the treatment with X-rays serves to emphasize the vesicular condition of the chromosomes to make more clear relationships which undoubtedly already existed. Miss Carothers finds that X-ray treatment tends to increase the ease with which she finds chromatin granules in the peculiar vesicular condition which she calls "chromosomal vesicles."[1] It is possible that the fact that I first studied the radiated material is accountable for the recognition of the conditions here, although having once worked out the facts there I had no trouble in verifying the observations in normal untreated and uncrossed *Fundulus* eggs. Many of the findings have been confirmed by Miss Pinney in a study of *Fundulus* eggs as yet unpublished, and I have demonstrated my slides to various workers at Woods Hole, none of whom had any difficulty in seeing the conditions here described.

This account offers a rather different history of chromosome behavior from that usually given. The transition stages from one step to another are very clear, however, and do not usually permit of any other interpretation, so far as the writer can see, than the one here set forth. The new account of chromosomal formation and processes connected therewith, it will be seen, is easily fitted into the orthodox interpretation without doing violence to any important conception of mitotic behavior. It is on the details of the transition from one phase of mitosis to another that the new conception obtained from the study of the processes in *Fundulus* throws light, and at the same time offers a "raison d'être" for the appearances seen.

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[1] This use of the term is unfortunate in the light of its earlier application in the sense used throughout this paper. "Chromosomal vesicle" in the sense of Sutton and Conklin refers to a vesicular condition of the whole chromosome. Miss Carothers has subsequently pointed out that the structure she described is in reality a plasmosome vesicle. (Jour. of Morph., 28, p. 465, 1917.)

Fundulus cleavage cells show nuclei which are favorable to the study of these processes for the very reason that they are small in relation to the large amount of cytoplasm present. Because of the physical conditions thus engendered, the chromosomes and chromatic structures have a characteristic loose arrangement which permits their close study. At the same time this renders a study of individual chromosomes, as has been done for instance in orthopteran material, impossible. The behavior of a group of chromosomes as a whole is easily followed, but individual differences as in size in metaphase chromosomes are so slight that it is quite beyond the range of practicability to follow a single chromosome through the various metamorphoses it undergoes.

The material used in this investigation consisted of *Fundulus* eggs, fertilized as already stated by either *Fundulus* or *Ctenolabrus* sperm. Eggs were preserved during the early stages of cleavage (first five cleavages), and also during the later stages of development, but the cells from the early stages are the most favorable for cytological study, and it is chiefly from them that the figures are drawn. Fixation was most satisfactory in Bouin, although other fluids were also used. Iron hæmatoxylin, variously counterstained, gave the best results. The blastodiscs were removed from the yolk and embedded and sectioned separately.

Observations.

The metaphase plate may serve as a convenient point at which to begin observations on the mitotic process. Fig. 1 shows the conditions of chromosome and spindle relation in the normal *Fundulus* eggs at the time of maximum condensation of the chromosomes in the metaphase. It is from a blastomere in the fifth cleavage division. The cytoplasmic mass of such a blastomere is quite large as contrasted with the volume occupied by the spindle, and of course is large in comparison with the amount of chromatin present. This figure shows conditions similar to those which led Van der Stricht to conclude that the nuclear sap passes to the poles of the spindle and is equally divided by the ensuing halving of the blastomere. The fibers are apparently grouped into bundles leaving spaces along which the clear streams of nuclear sap may flow to the poles. Van der Stricht regards this as evidence that the sap of the dividing nucleus gives rise to that of the daughter of nuclei.

In general the events which take place during the metaphase of mitoses in these eggs at least during the earlier cleavage divisions, do not differ greatly from those occurring regularly in the majority of animal and plant cells. It is with those phases of the mitotic cycle that follow the metaphase and continue until the stage of formation of the new metaphase is reached, that are of especial note here.

Fig. 2 shows the conditions obtaining at the moment of chromosomal division. Three of a group of chromosomes are here half divided and the ends are pulling apart preparatory to their separation in the anaphase. Already it is possible to detect the chromomeres of which the chromosome is composed. The metaphase represents the stage of maximum contraction of the chromosomes. It seems that at the moment of separation the chromosome begins to loosen up. This may be interpreted to mean that their permeability increases at this time; at any rate they take in liquid (water or cell sap?) resulting in the swelling up of chromosomes and consequent separation of the chromomeres. It is in this manner that a chromosomal vesicle begins its formation. As a result of this process the chromosomes lose their property of staining densely, and until they have passed well into the telophase, the walls of the vesicles thus formed retain the chromatic stain longest. As the vesicle grows, however, it is seen that the latter characteristic is really misleading, for the walls are merely lined with very fine granules of chromatin which later become separated by the increasing growth of the vesicle.

Fig. 3 is an anaphase. The loosening up process here has brought out the true character of the chromosome. A chromosome consists of two substances, linin and chromatin, of which the former is in the nature of a sheath or sac, while the latter exists as a mass contained in the sheath. Conklin has described a similar structure for *Crepidula* chromosomes which consist of "chromatin enclosed in a linin sheath." Figs. 4 to 7 show successive steps in the anaphase and the various steps in the process of vesicle formation. The chromomeres break up and the granules of chromatin become peripherally arranged, and the center is for a while free from granules.

This central cavity of the chromosome does not seem to be a definite vacuole, as is figured for instance in some plant chromosomes, but is simply the space within the walls filled in from the fluid portion of the cytoplasm. The vesicle is achromatic in its interior due to the superficial distribution of the chromatin. Gradually there appear very fine strands or fibrils, doubtless of linin, growing from the vesicle wall inward and the granules of chromatin which accompany them are particularly noticeable at the intersection of the fibrils. Later the fibrils become stronger and somewhat reticulated, while the granules on the inner wall are farther and farther apart, and the vesicle is itself in appearance a tiny nucleus in the typical resting stage. When the reconstruction of the nucleus is complete, the granules are small and fairly equally distributed and do not stain very densely. At no time, however, does the nucleus become completely achromatic as is held in some cases. The behavior of the chromosome during its reconstruction permits a very rough comparison to an elastic bag tightly packed tightly with a fine granular substance. As the bag takes in liquid it swells up and the non-soluble granular substance becomes rolled up into tiny balls on the inner surface, and occasionally chains of the granules extend into the interior.

This interpretation of the chromosome is not unlike Conklin's view that these bodies consist of chromatin enclosed in a linin sheath. As they move to the poles they are transformed into vesicles, the interior of which becomes achromatic, "though frequently containing a nucleolus-like body, while the wall remains chromatic. These vesicles continue to enlarge and then unite into a 'resting nucleus'; the nuclear membrane is composed of the outer walls of the vesicles, while the inner walls stretch through the nucleus as chromatic portions." The formation of chromosomal vesicles in the anaphases of ova has not been a matter of infrequent observation; and this phenomenon is not commonly seen in cells other than egg cells. In other words, the formation of chromosomal vesicles is, without much doubt, correlated with the presence of an unusually large amount of cytoplasm. As the chromosomes are transformed into vesicles, they absorb, it seems, large amounts of the achromatic material from the cytoplasm, by which they grow to resemble the familiar reticular structure. Their true nature becomes masked, but as will appear later, is not really changed. Vesicular chromosomes have been described in fish eggs by Moenkhaus and certain of the figures of Miss Morris indicate that she also saw them.

According to the usual interpretation, and to this Moenkhaus subscribes, the individual vesicles fuse with their neighbors and these larger ones with each other until at last the entire nucleus is simply one great vesicle. Moenkhaus describes the transformation of the chromosomes into resting nuclei as taking place by their conversion into vesicles which during an early stage can be distinguished into two groups by the difference in size. Then the smaller vesicles "fuse at first into larger ones, giving rise to a lobed nucleus. At this state it is no longer possible to tell the two kinds of vesicles apart. The fusion continues until a single well rounded nucleus results, with all traces of its double character lost."

Increase in size of the chromosome continues through the telophase stages and to the resting stage. A rough calculation of the growth from the stage (Fig. 7) where the definite vesicular character of the chromosomes (late anaphases and early telophases) is first discernable to the full size found in resting nucleus (Fig. 12) indicates a fivefold increase. It is scarcely possible to compare quantitatively the amounts of chromatin in the condensed chromosome and in the disperse vesicular stage. But one cannot easily doubt that an actual increase has taken place in the latter case. It is during this time in all probability that the new chromatin is formed in preparation for the ensuing division; and at the same time growth in the amount of achromatic substance present would also take place. The swelling up process must of necessity be reversed at the prophase to a certain extent, and some of the liquid which had been taken in be expelled; as will be described later some shrinkage does actually take place.

During the transformation as well as subsequent to it two kinds of vesicles can be distinguished on the basis of size in the D series, in the control, and in the A series. The larger ones represent the *Fundulus* chromosomes and the smaller ones those from *Ctenolabrus*. Since in these three sets of experiments the eggs and sperm were equally injured (or in the control, were equally uninjured) by the radiation, exactly that result was to be expected in keeping with the findings of Moenkhaus and Miss Morris. The B and C series have contributed little to this phase of the investigation, but the conditions, while more complex there, apparently offer confirmation of the fact discovered by Hertwig that chromatin from radiated nuclei does not take part in the spindle to any great extent after development is initiated; or, as they have described it, the development in a strict sense thus becomes parthenogenetic. It is not to be expected therefore that the sperm chromatin in the case of the radiated B series, nor the egg chromatin in the C series will make any considerable contribution to the development of these hybrids. Such condition is found to obtain at least so far as my study has extended.

Figs. 8 and 12 represent various nuclear changes during the telophases. The cytoplasmic constriction begins to cut into the cell at about the time of Fig. 8; Fig. 12 was drawn from a cell in which the division was practically complete. There is very little difference between the latter and the resting stage shown in the next figure. During the telophase, growth of the vesicle continues and the distribution of the chromatin granules takes place. Thus the vesicles become approximated to each other so that finally there remains no space between them and each has conformed to its neighbors in assuming its final shape. Yet at all times they can be seen to retain their distinctly individual character, and in well fixed material the outlines of each can be followed. The figures are inadequate to represent the conditions for they are drawn all at one level, and the advantage that careful focusing gives in studying their structure is lost. Some of the vesicles are more distinct than others, however, as is shown in Fig. 10, where one of them stands out with especial clearness. It may not be objected to these figures that they are tangential sections and that the vesicles really fuse in the center of the nucleus, for many of these nuclei extend through three sections and in each of the sections the same conditions are seen; if this condition were due merely to the tangential cut of the section the second of a series of three would not show the vesicles separately as it actually does.

Reconstruction consists in the further swelling up and rounding out of the anaphase vesicles and their close approximation to each other in the telophase. In the completely reconstructed nucleus the approximation of the vesicles is very close, so that they are no longer to be recognized as separated bodies. Yet the appearances do not indicate any actual fusion. One sees a nucleus consisting of vesicles so closely applied to each other that they conform in shape each to the other, and there are left no spaces between them. The inner boundaries are less sharp than the nuclear membrane, but even the nuclei which are only lightly stained show areas marked off from each other which represent the tightly packed vesicles. In every resting nucleus, whether radiated or not, which I have studied these areas can be made out. Fig. 15 represents a fairly typical case, although in other nuclei the vescular structure is even more apparent. That these vesicular areas do not represent simply a delay in the complete fusion can be seen by referring to Fig. 22 where their walls are still distinct, although the new chromosomes for the next division have been formed.

The resting condition of the nuclear vesicles is shown in Figs. 13, 14 and 15. The first two of these are from eggs before the first cleavage division; until they were found the writer did not feel sure that the vesicular condition represents more than a delay in the fusion and that the final result might be such as Moenkhaus has described. Since in these cases there had been no preceding division, however, and since it does not seem that the unfused condition would persist so long as from the maturation division and over the fusion of the pronuclei if the parts do not normally coalesce, these resting nuclei gave additional credence to the belief that the vesicles maintain themselves over the resting stage as separate entities. Subsequent observations on the prophase render this position indisputable. (See below in connection with Fig. 22.) Careful study under very high power with proper conditions of lens definition and illumination on well fixed material leave very little doubt that the vesicles do remain separate. It is true that a one-to-one correspondence cannot be established between the number of vesicles in the resting stage and the number of chromosomes in the metaphase plate. This is true for two reasons: it is not possible to count with accuracy the number of these chromosomes when they are most condensed and therefore have the sharpest outline; and it is even more impossible to count the number of vesicles, for the plane of the section usually passes through some of them, and it is too uncertain to attempt to superimpose the vesicles of one section on those of the other to make a count, while at the same time all of the vesicles cannot be seen in a preparation of the nucleus as a whole. The inability to count the number of vesicles, however, is hardly an argument against their permanence for the reason that they can be followed through the interkinesis and prophases as is shown in the later figures.

These observations on the method of the reconstruction of the nuclei have a certain bearing on the problem of the nature of the nuclear membrane. Here, at least in its origin, the membrane is formed of the outer walls of the chromosomal vesicles. It may well be that the nuclear membrane of the resting cell is more complex than this, due to the interaction between nucleus and cytoplasm; indeed, some hold that the outer part of the membrane is of cytoplasmic origin, and is therefore more of an inner limiting membrane to the cytoplasm than of a constituent part of nucleus. The writer sees evidence for this view on Figs. 24 and 25 where more or less of the nuclear membrane still remains, although the inner vesicular walls have broken down and the new chromosomes have already formed.

Nuclear membranes of the *Fundulus* type which are really the outer vesicular walls find a parallel in the membrane formation around single chromosomes which have become abnormally isolated, and have grown into small nuclei distinct from the true nucleus of the cell. Such a case Boveri described for *Ascaris*. Except in regard to size and the number of chromosomes entering into them, these smaller nuclei have the same structure as those of regular types, and their membranes cannot be distinguished from that of the larger nucleus. Boveri's minute pathological nuclei are themselves an argument for the view that the chromosomes retain their independence in the normal completely fused nuclei which is usually described for this form. In these hybrid fish eggs, fundamentally the same condition obtains, for the vesicles, closely applied to each other as they are, actually retain their identity, although no cytoplasmic layer intervenes between them. Vedjdowsky has expressed a somewhat similar view in regard to the nuclear membrane for he holds that it represents the peripheral portion of the certain chromosomes that have become vacuolated in their interior.

Experimental vesiculation of chromosomes has not proven difficult to bring about in cleaving eggs. Years ago Boveri published the results on *Ascaris* eggs just mentioned, in which the chromosomes may fail to fuse and each form its own nucleus-like vesicle, and his observations have been repeatedly confirmed. Conklin's numerous experiments on *Crepidula* eggs ('12) will suffice to show how common a phenomena vesiculation is. By various means the chromosomes of the eggs were prevented from fusing and each formed in the resting stage an independent "karyomere" quite comparable to Boveri's cases. Other instances might be cited.

A phenomenon which may be so easily induced as this one is most readily understood if one thinks of the normal nucleus in interkinesis as consisting of closely crowded entities, the vesicles, derived from the telophase chromosomes, which never normally coalesce. This means that the unusual conditions of the experiment merely push them a little apart from each other. As in the case of a catalyzed reaction, a condition which already exists may be modified easily, while it is very difficult indeed to bring about an entirely new set of relationships between elements not normally reacting.

Conklin has developed the idea of karyomeres, or chromosomal vesicles, basing it upon his observations and experiments, to such a stage of completion that he can show all stages of combinations from single vesicles on up to that of the entire complex. He figures cases that very closely resemble amitosis as shown by various workers, and suggests that this superficial resemblance may be a source of error in some at least of the observations upon which the claims of the occurrence of amitosis have been made. This suggestion may be reiterated here, for it is obvious that if there should be an indention between two of the vesicles in the resting stage of a *Fundulus* nucleus, direct division would be at once called to mind.

The polarity of the cell is definitely maintained in dividing *Fundulus* eggs. As the chromosomes pass to the poles their long axes lie in the cell axis, which passes through spheres and nuclei, and the vesicles are formed with this polarity. Cytoplasmic division follows only slowly after nuclear division and the nucleus has entered upon the phase of interkinesis by the time that the cytoplasmic separation is accomplished. Also before cytoplasmic separation, the centrosome divides and each half traverses an arc of 90° in preparation for the next division. This results in the establishment of a new cell axis at the right angles to the old. Thus for a time the old and the new axes exist together, the daughter nuclei themselves marking the old and their divided centrosomes the new. Fig. 16 is such a case, the two cells shown having arisen from the result of the division of an ectodermal cell of a blastula. The line connecting the points *A* and *B* represents the axis of the parent cell, while *CD* and *EF* respectively mark the axes of the daughter cells. The fact that the two latter are nearly perpendicular to each other is of significance when it is remembered that in the next division the upper of these cells will produce the two ectodermal cells and the lower two mesodermal.

The polarity is recognizable in the vesicles in many cases, the longer axes of the vesicles in general lying in the cell axis. It is, therefore, evident that about the time of centrosomal separation the polarity of the vesicles changes. Earlier oriented with respect to the old sphere, they have now shifted to the newly established axis. Whether this is by an actual revolution of the entire vesicle, or only by a change of shape so that its short diameter now becomes its long axis is not clear, but it seems probable that the former process takes place. When condensation occurs and the new prophase chromosomes are formed they show the polarity in a very striking manner, almost every chromosome in the spireme stage being oriented in the long axis of the forming spindle (see Fig. 22). This of course is to be expected in view of the definite polarity of the vesicles in the prophase as shown in Fig. 17.

In reconstructing nuclei where the fixation is not quite perfect, the walls of the vesicles sometimes cannot be seen. They are very delicate and require a very complete fixation if they are to be preserved in their entirety. When the nucleus enters upon the first steps of the prophase and liquid is extruded from the vesicles so that they shrink or condense, the walls are particularly difficult to preserve. This results in an artificial running together of the granules into irregular clumps. Yet the area formerly occupied by the vesicle is still to be seen separating one clump of chromatin from the next, and the nuclear membrane is still retained although in a shrunken condition. The chromatin so massed by the irregular plasmolysis of the walls of the vesicles in some instances comes to resemble a prophase chromosome. Mere clumps of chromatin granules, however, with nuclear membrane intact and the inner vesicle walls gone indicate artifacts rather than normal conditions. Yet even they serve to suggest certain facts which are represented in the normal nuclei.

The new chromosomes arise within the old vesicles. When the writer first began the study of this phase of the subject, he was led to expect that the shrinkage which takes place at the beginning of the prophase as shown in Figs. 13, 17, and 18, would continue and the formation of the new chromosomes would be simply the reverse of the process by which the vesicles had been formed. Such is not the case, however, for it is now certain that the new chromosomes arise endogenously. The formation begins within the vesicle with the gradual increase in size and the aggregation of the chromatin granules upon its walls. The typical condition of the vesicles during rest shows small granules distributed fairly uniformly throughout it. Succeeding this stage of general chromatic dispersion the vesicles appear to shrink, expressing part of their water content; this phenomenon is manifested doubly, first by the clear non-fibrillar area about the nucleus, the extent of which before shrinkage is thus marked, and second by the concentration of the contents of the vesicle, which in fixed material appears denser and takes more orange G than before. Coincident with this change the amount of chromatin appears to increase and the aggregation of the granules takes place, the larger masses of chromatin appearing along the periphery and perhaps extending into the center along a single thread. The aggregation continues until masses the size of the chromomeres of the anaphase are formed and have become arranged in a linear series. The linin sheath about this linear aggregation of chromomeres forms early. This structure is the new chromosome and must be thought of as chromosomal in nature quite as much as the more familiar metaphase stages. When it is completely formed the walls of the vesicle may be seen to be gradually dissolved and the denser contents to diffuse into the cytoplasm. About this time or a little later the split of the new, or filial, chromosome takes place, each chromosome and chromomere showing it about equally.

Figs. 17 and 18 show the first sign of the next division. The chromatin granules are well dispersed and the vesicles definitely oriented, while the pre-mitotic shrinkage has taken place. In Fig. 19 the aggregation of the chromatin granules has begun and the vesicles in this state can always be recognized as about to divide. In Fig. 20 is seen the linear arrangement of the chromomeres, a process which is well advanced in Fig. 21. In this latter case indeed the chromosomes are well formed and the linin sheaths can be made out. In these figures the walls of the vesicles are not as clear as in some cases, but the denser gel-like portion still shows the form and relationships of the vesicles. There would seem to be some variation as to the exact time when the disappearance of the walls takes place, in relation to the development of the spindle structures at least.

Figs. 21 and 22 are prophases in the spireme stage, early and later. Fig. 22 is an especially clear case showing the newly formed filial chromosomes while at the same time the walls of the old vesicles have not yet disappeared. In the upper part of this figure they are less distinct, but elsewhere the vesicles are easily seen in their entirety. The walls stand out clearly and take the orange stain strongly, so that there can be no mistake about their presence. The spheres are not shown in the plane of this section, but the spindle fibers indicate the polarity with most of the chromosomes oriented in the spindle axis.

Of the greatest importance is the evidence presented in Fig. 22. The presence in the vesicles, the walls of which are still intact, of the new filial chromosomes, for these spireme structures are now definite chromosomes, is the crux of the argument for chromosomal continuity. It is not difficult to follow through the history of the vesicles from the time of their earliest formation out of the metaphase chromosomes thorugh anaphases, telophases, and interkinesis, and to see that the vesicle represents, so far as the chromosomal bodies are concerned, a continuous unit of structure. The parental chromosomes are not lost in the vesicle stage; they are merely metamorphosed, appearing in a new form and the chain of the continuity is never broken. In the vesicles the continuity is still maintained throughout the resting period of the nucleus and it is only in the late prophase of the daughter generation that the vesicular walls disappear. But before that disappearance, the new filial chromosomes have been formed and are in the so-called spireme stage, representing uninterruptedly the persistent continuity of the units with which the cycle started in the parental metaphase. In this spireme stage the chromosomes are as definitely chromosomes as in the metaphase which immediately succeeds, and they have only to condense further and to attach themselves to the spindle to enter the metaphase, a process obviously favored by the dissolution of the nuclear walls.

In Fig. 23 all traces of the vesicle walls have disappeared and the chromosomes resemble the late prophase chromosomes of many animals. This is the only case in which the longitudinal split is seen, and it may be that it is really precocious, or that the figure represents the facts only in part. In most nuclei in which the chromosomes are well formed the spindle development has progressed farther than in this case. These split chromosomes are suggestive, however, when compared with such chromosomes as shown in Fig. 2.

The manner of attachment of the fiber to the chromosomes and the steps leading to the metaphase plate condition are shown in Figs. 24 and 25. Fig. 24 is an oblique section in which there is only one sphere. The walls of the vesicles at the polar end of the nucleus are gradually disappearing and the fibers are making their way to prophase chromosomes; traces of the walls however can yet be seen. In the next figure the attachment of the fibers is practically completed and the chromosomes have become definitely placed upon the spindle. While in general they were orientated in the prophase with their long axis in the spindle axis, they become definitely "drawn" into place with the attachment to the fibers. The condensation of the chromosomes and their final arrangement on the spindle completes the cycle and brings us again to the stage shown in Fig. 1.

These last two figures are interesting also for their likeness to the conditions found by Van der Stricht in the egg of *Thysanozoön*. In this form the spindle and its fibers can be differentiated into several parts. The principle cone, "cone principaux," of fibers corresponds to the mantle fibers and is composed of those which attach to the chromosomes themselves. Just outside these but still in the area of the nucleus is a cone of fibers which do not attach; this Van der Stricht called "cone accessoire." Finally out in the cytoplasm is to be seen a series of the fibers which interlock with those from the other end of the spindle; these are the "fibers cytoplasmique." In *Fundulus* eggs exactly the same conditions seem to prevail (Figs. 25 and 1).

As the central spindle fibers make their way to the chromosomes and attach to them there is a gradual movement of the chromosomes to the equator of the spindle to form a plate. During this movement the last stages in the condensation of the chromosomes takes place, resulting in the formation of the densely staining metaphase rods, such as were figured by Moenkhaus and Miss Morris. As they condense the chromosomes increase in thickness, so that the metaphase chromosome is both shorter and broader than that of any other stage. The nuclear sap also becomes completely diffused into the cytoplasm by this time, and no distinction can be seen. The final event leading up to the formation of the plate is a change in position of the chromosomes with the reference to the axis of the spindle. Previously oriented with their axes parallel to that of the spindle, they now come to lie at right angles with their free ends pointing towards the periphery. When this stage has been reached the plate is fully formed.

Thus it is possible to follow out the history of the chromosome group in the early cleavages of *Fundulus* without a single break in the continuity from the metaphase of one generation to that of the next. It should perhaps be pointed out again that this account refers to no particular mitosis. In all of the early cleavage divisions in which the nuclear structures are large enough for study the facts are as here related.

Since making the above observations on *Fundulus* the writer has been able to extend them to many stages of the eggs of *Corregonus albus*, and to satisfy himself that the behavior of nuclear structures is essentially similar in both fish.

Figs. 26 to 29 do not add any direct proof to the theory of the continuity of the chromosomes, but they serve to throw a side light on the process of spindle formation, at least as it occurs in the gastrulæ of this form. The writer has not seen the same conditions in the early cleavage cells and does not presume to claim that they occur there, but since the gastrula shows these rather unusual phenomena, they may be set forth here to assist perhaps in the interpretation of similar appearances elsewhere. At the beginning of the prophase, the formation of the central spindle takes place between the centers that are here divided and are passing to the opposite side of the nucleus. This process, it will be noted, occurs at a relatively later period in the division of the gastrula cells than in the earlier cleavage cells. Fig. 27 is a face view of a spindle of this type a little farther developed; certain of the chromosomal vesicles become separated to make way for the developing spindle which gradually sinks down between them and is thus ready for the attachment of the fibers to the chromosomes whenever the latter are formed. It might be thought by analogy with such forms as *Crepidula* that

Fig. 27 really represents the male and female halves of the nucleus. That this is not the case, however, is shown by Fig. 28 in which only the *Fundulus* chromosomes are visible. Miss Morris found that in *Fundulus* cytoplasm the *Ctenolabrus* chromosomes are small round bodies, distinctly differentiated from those of *Fundulus*. This observation I can corroborate; the cytoplasm modifies the character of the foreign chromosomes, and they can be recognized as different from those of the egg. The egg from which these four figures were drawn was fertilized with *Ctenolabrus* sperm that had been subjected to X-radiation for fifteen minutes; this is sufficient duration of time for the radiation to kill the male chromatin, which therefore made no contribution to the development of the egg and is not represented, as is shown by Fig. 28 which contains only *Fundulus* chromosomes. Since there is no male contribution to this nucleus, the interpretation that Fig. 27 shows merely the male and female halves of the nucleus is not valid. Rather the true state of affairs is made clear by Fig. 29 which is a cross-section of such a nucleus as is shown in Fig. 27; the central spindle makes its way through the groove shown on the upper side of the nucleus.

Discussion.

(*a*) *Chromosomal Vesicles.*

While there are numerous cases cited in the literature which would seem to give the conception of the constitution of chromosomes and resting nuclei here set forth a fairly wide application, the writer does not care to attempt a generalization from these observations extending the principle to other forms, although some of the cases which support these findings must be given. Any generalization at this time would seem unwarranted for too many instances are known where neither the chromosomes nor the chromatin during rest seem to answer exactly to these descriptions. There are many cases, for example, in which the chromosomes, judging from their behavior in the prophases, may be regarded as made up in chromatin granules strung upon a linin thread, and it is even possible to identify the granules in homologous chromosomes. How such a structure is to be derived from a permanent vesicular chromosome is not yet clear, although this derivation is perhaps thinkable. The account here given aims rather merely to present the conditions as they are found in the fish eggs which I have studied, and to point out that these facts may have significance for current cytological and genetic speculation.

Conklin in his studies on *Crepidula* (1902) has perhaps followed out the history of the chromosomal vesicles, or karyomeres, as he calls them, more completely than any other observer. Of the behavior of the *Crepidula* chromosomes he says: "In large cells where the divisions succeed one another at short intervals the chromosomes begin the growth characteristic of the daughter nuclei, the absorption of substances from the cell body, before they have fused together, whereas in small cells or cells which divide only at long intervals the chromosomes fuse before the absorption of achromatic material begins."

"The history of the nuclear changes during the cycle of division may be summarized as follows: (1) The chromosomes, consisting of chromatin enclosed in a linin sheath, divide and move to the poles of the spindle where they partly surround the spheres. (2) Here they become vesicular, the interior of the vesicle becoming achromatic, though frequently containing a nucleolus like body, while the walls remain chromatic. (3) The vesicles continue to enlarge and then unite into the 'resting nucleus'; the nuclear membrane is composed of the outermost walls of the vesicles, while the inner walls stretch through the nucleus as chromatic partitions; the chromosomal vesicles from the egg and sperm nuclei remain distinct longer than those from the same nucleus. (4) The chromatin of the inner alveolar walls then aggregates into threads, giving rise to a 'chromatic reticulum,' though the linin still preserves, for a time at least, the alveolar structure. (5) The chromatin of these threads then separates into spherules, which are connected together by linin threads; these spherules vary in size, and at first are solid, and stain alike. (6) They become hollow and are differentiated into oxy- and basichromatin. (7) In the first maturation, each of the basichromatin spherules, or bodies, grows into an individual chromosome; in the cleavage, the basichromatin spherules unite into several linear series, thus forming a segmented spireme.

(8) The oxychromatin spherules grow smaller and are dissolved in the nuclear sap while others are arranged in series on the linin threads into which they are formed; these threads with attached spherules form the spindle fibers." Thus the relation of the old vesicle to the new chromosome is followed out.

Evidently from this description there is a close parallel between the behavior of the chromosomes of *Crepidula* and those of *Fundulus* eggs. Much of this summary will fit, word for word, the conditions which have been delineated in the preceding pages, although in *Fundulus* the vesicles do not fuse in the resting stage.

Conklin points out the fact that "such vesicles are found generally, if not uniformly, in the early divisions of the ova, though they are not usually found in other mitoses." He attributes this to the difference in size and rapidity of division of the blastomeres as compared with tissue cells, and concludes "that in the large cells where divisions succeed each other at short intervals, the chromosomes begin the growth characteristic of the daughter nuclei, *i. e.*, the absorption of substances from the cell body, before they have fused together, whereas in small cells or cells which divide only at long intervals the chromosomes fuse before the absorption of achromatic material begins." With this conclusion the facts as found in *Fundulus* in general are in accord.

In both kinds of eggs also the growth stages of the daughter nuclei are quite alike. In *Crepidula* "after the fusion of the chromosomal vesicles to form the daughter nuclei, the latter continue to absorb achromatic material, growing larger and larger until the prophase of the next division. A part at least of the achromatic material absorbed is derived from the sphere which in turn contains interfilar substance of the spindle and aster. This recalls the conclusions of O. Hertwig in which he points out that in the formation of the daughter nucleus the chromosomes absorb 'Kernsaft' and become vesicular, the process being the reverse of what occurs in the beginning of division where 'Kernsaft' is set free into the cell body. A similar view was held by Butschli . . . ."

The most important point of difference between Conklin's observations and mine is the matter of ultimate fusion of the chromosomal vesicles in the resting stage, and the formation of the new chromosome in the vesicle. In *Fundulus* there can be no doubt that a direct continuity exists between vesicle and prophase chromosome and that the vesicles do not fuse during the rest period. One is compelled to suspect that *Crepidula* offers less favorable opportunity for the study of this particular detail than *Fundulus.* Conklin himself believed that there is a real independence of the vesicles although they appeared to fuse, and it may be inferred from the following paragraph that he expected their persistent identity throughout the resting nucleus to be proven in time. The observations on *Fundulus* may be taken as a confirmation of his view expressed in 1902.

"It sometimes happens, especially in eggs in which more than the normal number of centrosomes and asters are present, that some or all of the chromosomal vesicles do not fuse but remain distinct through the whole resting period. In such cases each of the vesicles behaves like a minature nucleus, absorbing the achromatic material and forming a network of chromatin either within the vesicle or on its walls. In this growth and differentiation the vesicle keeps pace, step by step, with the normal nucleus, so that one must regard the resting nucleus as virtually composed of vesicles, though their union may be so intimate as to hide this structure. The resting nucleus is not, therefore, a single structure any more than is the nuclear plate. It is composed of units each of which so far as known, has the properties of the entire nucleus, and the union of these vesicles into a single one may be considered as a secondary character. It is altogether probable that the chromosomes and hence the chromosomal vesicles, preserve their identity throughout the resting nucleus."

Moenkhaus also was unable to see in the nuclei of the resting cells evidences of separate chromosomes, yet he too, believed that the substance of each chromosome forms a persisting unit. We may expect then that the next step in the proof of chromosome continuity and persistence is the recognition of structures in the resting nuclei which can be homologized with the chromosomes of the metaphase plate. This I believe is accomplished in the fish eggs of my experiments. Moenkhaus states the case as follows: "The question whether the individual chromosomes persist through the resting stage so that upon the resolution of the reticulum into the chromosomes the same component chromatin granules again go together to make the same chromosomes from which they were derived is a question first raised by Rabl and later definitely stated by Boveri. Since that time so much evidence has accumulated to support this conclusion that it has come to be rather generally accepted. Even a general review of the evidence is unnecessary here. Such a review would show that the fact has never been definitely demonstrated. Some of the most direct evidences yet given are the observations of Herla and Zoja on the *Ascaris* hybrids in which it was shown that the small chromosomes of the variety *univalens* which entered the resting nucleus with the larger ones of the variety *bivalens* again emerged in characteristic form. Equally strong evidence is now afforded by my observations on hybrid fishes. Here, as in the *Ascaris* hybrid, two kinds of chromosomes enter the resting nucleus from which each kind again emerges. As long as the two kinds remain grouped, as during the two divisions, this fact has little added significance, since within each group it would be perfectly possible for the component chromosomes to exchange chromatin granules during the resting period. If, however, as occurs in the later cleavages, the two kinds of chromosomes become mingled the chromatin granules of both kinds must lie mingled together within the resting nucleus. If from a nucleus the two kinds again emerge, it amounts almost to a demonstration that the chromatin substance of a given chromosome forms a unit and that the unit persists."

The evidence presented by Conklin and by Moenkhaus most nearly touches the subject of my investigation of any that I have found in the literature. Both of these workers failed to see in their material that the chromosomes may actually be discerned in the nuclei during rest as distinct vesicles. Yet both believed that a continuity of structure does exist and that the chromosomes form persistent, independent units. My material offers more complete and direct evidence that the vesicles persist and leaves little room for doubt that the conclusion of the observers mentioned is the correct one.

Sutton (1900) was the first of a group of cytologists working on the chromosomes of grasshoppers to point out that in the early stages of nuclear formation each chromosome forms a vesicle about itself, but thought that later the proximal ends of these fused, a condition which he interpreted as supporting the idea of chromosome individuality. His conception of a chromosome is not unlike that of Conklin, and has since been amply confirmed.

The occurrence of the chromosomal vesicles is quite common in the eggs of animals, and it has also been reported in certain other tissues as well. The following list is presented merely to give a few examples; it is in no way complete, but will serve to show the wide distribution of the phenomenon.

Name, W. G. Von, Trans. Conn. Acad., Vol. X., 1899.	*Planorbis*, early development of eggs.	Figures vesicles in anaphases.
Goldschmidt, R., Zeit. f. wiss. Zool., Bd. 71, 1902.	*Polystomum* eggs.	Karyomerites, at first one to a chromosome; later fuse and are not recognizable.
Von Kemnitz, G. A., Arch. f. Zellf., Bd. 10, 1913.	*Branchycœlium* eggs.	Karyomerites which later fuse.
Grille, K., Arch. f. Zellf., 12, 1914.	*Gyrodactylus elegans* eggs.	Karyomerites which fuse later.
Boveri, Th., Merkel u. Bonnet's Ergebnisse, 1891.	*Ascaris* eggs.	Accidentally separated chromosomes produce vesicles, from which new chromosome arises.
Lillie, F. R., Jour. Exp. Zool., 12, 1912.	*Nereis* eggs.	Figures vesicles in telophase; later fuse in reconstruction.
Mead, A. D., Jour. of Morph., 13, 1897.	*Chætopterus* eggs.	Figures show what are probably chromosomal vesicles.
Lefevre, G., Jour. Exp. Zool., 4, 1907.	*Thalassema* first cleavage.	Vesicles in anaphases; later they fuse.
Wilson, E. B., The Cell, 1900.	*Toxopneustes* egg cleavage.	Figures vesicles in telophase.
Boveri, Th., Zellstudien, IV., 1900.	*Echinus* eggs.	Figures vesicles in anaphase.
Buchner, P., Arch. f. Zellf., 6, 1911.	Sea urchin.	Karyomerites in first parthenogenetic maturation division.
Konopacki, M., Arch. f. Zellf., 1911.	Sea urchin.	Vesicles produced experimentally; thinks of them as nuclear budding.
Schaxel, J., Arch. f. Mikr. Anat., Bd. 76, 1911.	*Strongylocentrotus* egg.	Vesicles in telophase; they fuse, then alveolize, producing fine nuclear network.
Bury, J., Arch. f. Entw., 36, 1913.	Echinoid egg cleavage.	Karyomerites in early stages.

Smallwood, M., Morph. Jahrb., 33, 1905.	Nudibranch eggs.	Telophase vesicles which later fuse.
Lillie, F. R., Jour. Morph., 17, 1901.	*Unio* eggs.	Figure of resting nucleus yet in reconstructing process shows vesicles.
Conklin, E. G., Jour. Phila. Acad. Nat. Sci., 15, 1912.	*Crepidula* eggs.	Experimentally produced karyomeres from single chromosomes.
Häcker, V., Arch. f. Mikr. Anat., 46, 1895.	*Cyclops* eggs.	A few small vesicles which are male and female contributions.
Ruckert, J., Arch. f. Mikr. Anat., 45, 1895.	*Cyclops* eggs.	Chromosomal vesicles in both male and female halves, in transition to resting stage.
Kühn, A., Arch. f. Zellf., 1, 1908.	*Daphina* parthenogenetic eggs.	Vesicles in telophase, but they do not prove continuity for they are unrecognizable during rest.
Amma, K., Arch. f. Zellf., 6, 1911.	*Cyclops* eggs.	Figures vesicles in four-cell stage.
Bigelow, M. A., Bul. Mus. Comp. Zool., XL., 1902.	*Lepas* eggs.	Figures show vesicles in early cleavage stages.
Sutton, S. S., Kans. Univ. Quart., 9, 1900.	*Brachystola* spermatogenesis.	Chromosomal vesicles which became fused at one end.
Otte, H., Zool. Jahrb., 24, 1907.	*Locusta* spermatogenesis.	Chromosomal vesicles which were thought to remain distinct.
Davis, H. S., Bul. Mus. Comp. Zool., 53, 1908.	*Locustidæ* and *Acrididæ*, spermatogenesis.	Vesicles fuse at one end.
Pinney, E., Kans. Univ. Sci. Bul., 4, 1908.	*Phrynotettix* spermatogenesis.	Vesicles which were thought to remain distinct through resting stage.
Wilson, E. B., Jour. Exp. Zool., 13, 1912.	*Oncopeltus* spermatogenesis.	No rest between maturation divisions; chromosomes crowd together, like vesicles, without loosening up; never fuse.
Wenrich, D. H., Bul. Mus. Comp. Zool., 60, 1916.	*Phrynotettix* spermatogenesis.	Vesicles retain their own limits in the nucleus in rest.
Medes, G., BIOL. BULL., 9, 1905.	*Scutigera forceps* spermatogenesis.	Vesicles in second spermatocyte; they fuse; but traces are often long retained.
Conklin, E. G., Jour. Phila. Acad. Nat. Sci., 13, 1905.	*Cynthia* eggs.	Vesicles in cleavages.
Moenkhaus, W. J., Amer. Jour. Anat., 3, 1904.	Eggs of *Fundulus* and *Menidia* crosses.	Vesicles which fuse, but believed to be distinct though invisible as such in rest.
Morris, M., Jour. Exp. Zool., 16, 1914.	*Fundulus* and *Ctenolabrus* crosses.	Vesicles which fuse.

Hertwig, G. & P., Arch. f. Mikr. Anat., 84, 1914.	*Gobius* and *Crenilabrus* crosses.	Vesicles in telophases which are thought to be signs of degeneration.
Van der Stricht, O., Arch. de Biol., 12, 1892.	*Triton* eggs.	Vesicles formed by looping of chromosomes; figures of resting nuclei suggest traces of vesicles.

(*b*) *Chromosomal Continuity.*

Chromosomal individuality has been so often argued of late years that a renewal of the discussion seems an almost trite and needless repetition. However, in the light of the facts here brought forth it may not be out of place to review the present status of the question. It must be understood that no argument is made that chromosomes are directly transmitted from one cell generation to the next as *identities;* that conception has long since been abandoned for it has been shown to be inadequate. Rather the argument is that the new chromosomes arise from the same structural substance as the old, a theory of genetic continuity. One prophase chromosome and only one arises from the substance of a single one from the preceding division. It is such a theory which is the subject of this discussion.

The hypothesis of chromosome individuality dates back to Rabl ('85) who "concluded that the chromosomes do not lose their individuality at the time of division, but persist in the chromatic reticulum of the nucleus" (Wilson). He thought that the disappearance of the chromosome in the reticulum was only apparent, for he could recognize in the reticulum of the salamander nuclei portions corresponding to the chromosomes. The reticulum is formed by the outgrowth of processes, secondary and tertiary, from the chromosomes which while fusing on the neighboring chromosomes, nevertheless, maintain their own identity.

That Rabl's view regarding the persistence of the chromosomes has not found general confirmation is indicated by the following quotation from Wilson, who, in discussing the theory of chromosome individuality[1] remarks "that in vast majority of cases the identity of the chromosome is wholly lost in the resting nucleus and the attempts to identify them through the polarity or other morphological features of the nuclear network have, on the whole, been futile. It is, therefore, an abuse of language to speak of a persistent individuality of the chromosome."

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[1] The Cell, p. 300.

There is, however, a great mass of evidence in favor of a view of chromosome individuality or rather, since there is so little evidence of actual chromosome persistence as unchanging and identical bodies during the resting stage, the hypothesis of genetic continuity of chromosomes. The fact that almost invariably the same number of chromosomes emerges from the resting nucleus that went into it, Boveri's studies of abnormal variations in the early development of *Ascaris* eggs, Zur Strassen's observations of the giant embryos of *Ascaris*, the previously mentioned discoveries of the independence of maternal and paternal chromatin in hybrid eggs, the constant recurrence of chromosomes in the same form and even position, *e. g.*, in root tip cells of *Yucca* (Clemens Müller) and in *Ascaris* eggs (Boveri), their gradation in size invariably repeated in certain insect germ cells, the history now well known of the accessory chromosome, which retains its identity from one cell generation to the next, the recent studies which have shown clearly the homologies of chromosomes other than the accessory as well as their independent behavior, and finally the correlation of the results of recent studies in genetics with such a behavior on the part of the chromosomes, all these points constitute strong inferential evidence in favor of a theory of chromosome continuity.

Chromosome continuity is a theory as applied to resting nuclei only. In the mitotic stages the facts are clear; the chromosomes are distinct bodies which behave as units and it is a general observation that those of one cell are homologous with those of others in the same organs and in general in the same species. It is only in the interkinesis that the facts partake in any way of the nature of a hypothesis and admit of differences of interpretation regarding the point here discussed. In this state it is difficult or impossible to recognize chromosomes as distinct entities and accounts which seek to distinguish them throughout rest have been and must be received with great caution. Adverse criticisms of the theory have been based upon claims of faulty technique, faulty observation, upon obvious objections to a hypothesis of strict individuality (which is impossible both in a chemical sense where two phases exist in the same cell, and where there must be interaction between the constituent parts of the system, and in a mathematical sense of unchangeableness) and to the more deeply grounded objection that the process is one of dynamic character and is not subject to morphological explanation.

Mathew's objection is perhaps the most significant of the first group. He says "All so-called nuclear stains of basic nature except the mordanted stains as iron hæmatoxylin combine with nucleic acid. Cytologists in following chromatin and chromosomes may be following only the inert skeletal material of the nucleus while the active albuminous material is entirely neglected since it does not gel or stain with basic dyes." Yet significance must attach to the *constant recurrence* of these structures whether they are of skeletal nature or otherwise, and to the fact that chromosome structures reproduce themselves and have done so indefinitely. If they are not more than skeletal in nature, they are at least the manifestations of the "protein or basic" and perhaps more active parts of the cell, and the correlation is so close that their behavior is no less significant.

Fick's objections as Wilson has pointed out refer only to the strict interpretation of individuality which is not supported by the evidence and which is no longer held by cytologists in general.

With regard to the morphological method of attack as one means of studying dynamic problems more is to be said in defense than against. The nucleus is, indeed, "a dynamic system," as one of the chief critics of the continuity hypothesis has pointed out; and the process by which it reproduces itself is of all biological phenomena the best example of a dynamic process. Yet a dynamic process whose basis is not material, indeed not morphological, is only with difficulty thinkable. In whatever may be the final terms of our thinking, chromosomes, chromomeres, molecules or ions, we deal in the end with structure and configuration; and this fact must make us hesitate in any attempt to detract from the significance of chromosome structure and continuity. We may believe consistently that the chromosome is a continuing structure, and that its correlations with processes of heredity are even so close as to be causal, without forgetting that its functions and behavior are dynamic. Child has pointed out with great propriety that "instead of being the basis the chromosome is itself a problem of heredity." We no longer think of chromosomes as ultimate units of structure or the unreducible cause of heredity processes. It is true that the heredity processes have been merely pushed farther back into the cell for explanation; but even then, a distinct step has been taken if the correlation can be traced as in *Drosophila*, between body characters and chromosomes; and a more complete dynamic explanation of the processes, along with that of chromosome structure and behavior, can only be eagerly awaited. Dynamic processes are of course at the basis of all biological functions; with the physical structure of the organic body they do their work, and new facts, if real facts, must be comprehended in the larger dynamic system. Biology is now well launched out in the attempt to find dynamic solutions to its problems, and morphology including cell morphology still has contributions to make to the attempt. It is difficult indeed to understand the structure of a mechanism without some knowledge of its function, but it is equally difficult to appreciate a structureless function.

Of interest from the standpoint of chromosomal independence are the observations now being carried on by Chambers on the micro-dissection of the living cell. It appears from this work that there is gradually being established from the living material a confirmation of the general principles of cell structure and behavior as worked out on fixed preparations. The fact that certain structures as polar radiations, are not visible in the living cell and are present in fixed cells, perhaps due to injury or precipitation caused by the fixing process, is not an argument against their significance since they occur constantly and in precise relations to other structures. If not significant in themselves they are at least manifestations of processes which lie more deeply in the cell. Of particular interest here is the observation as yet unpublished of Dr. Chambers, of which he kindly permits mention to be made here, that in grasshopper spermatocytes the telophasic chromosomes are to be found in the vesicular condition. When single grasshopper chromosomes are removed and allowed to grow in plasma they develop into round vesicles which by puncturing with the needle can be made to collapse.

From the work of many cytologists there has gradually accumulated a great mass of inferential evidence for chromosomal continuity which is well known, and the general nature of which was mentioned earlier in this paper. The form and size relations of chromosomes, the behavior of the sex chromosomes, which can be followed from one generation to the next, the homologous character of somatic chromosomes, certain chromosomes not correlated with sex (according to the work reported by a number of investigators, *e. g.*, by Wilson and by Carothers), all of these facts can be understood only with the greatest difficulty on any other basis than that of continuity of structure. A very recent case is Wenrich's finding that in *Phrynotettix* there exists a pair, "B," whose architecture is constant for all individuals studied, and another pair, "A," which is recognizable "through all stages from spermatogonia to spermatids." This case is very clear and of great importance.

Vejdovsky's ingenious theory is of interest in relation to my observations, although I do not find that the chromosome behavior of *Fundulus* tallies with his descriptions of observations upon which he bases his theory. He holds that the old chromosomes produce anlagen from which the new ones arise. As each organic individual comes from the anlage in the female organs, so do the chromosomes also arise from anlagen. The old chromosomes produce only anlagen for the new generation and the formation of the latter sets in with the beginning of the new cell. This anlage is the chromonema which gives rise to the two substances of which chromosomes consist, chromatin and linin. Each chromosome begins its existence with a spiral chromatin thread while the linin substrate of the old thread is lost to the anlage of the "Kernenchylem."

Reference must be made to the bearing of genetical work on the doctrine of the significance of chromosomes. The results of the great mass of recent work in this field demands as a practical necessity that chromosomes be treated as if they are actually persistent individuals, and the genetical behavior of heredity characters is being steadily brought into line with chromosomal behavior as a more thorough-going knowledge of the latter is obtained. It is a noteworthy fact that those cases where heredity does not seem to conform to the expectation based upon the behavior of chromosomes are usually cases where the chromosomes are small, numerous and imperfectly known, and that in general the difficulties in comparing the two sets of facts have to a large extent disappeared with increasing knowledge.

The significance of hybridization experiments for the doctrine of chromosome continuity has been repeatedly pointed out, and a single case will serve to illustrate it. Federley crosses different species of the moth *Pygæra* in which the diploid and haploid chromosome numbers are as follows: *P. anachoreta*, sixty and thirty; *P. curtula*, fifty-eight and twenty-nine; and *P. pigra*, forty-six and twenty-three. In whatever cross was made of these three species the chromosome number of the hybrid was the sum of the two haploid sets that went in. According to Federley the chromosomes preserve their continuity through many generations of foreign cytoplasm.

Evidence that there is chromosome continuity in plants is quite abundant, as may be seen for example, from the reviews of Stout and of Wenrich. Only the main trends of the evidence need be mentioned here. First of these are the findings of Rosenberg, Overton, Lundagård and others that in the somatic cells of a considerable number of plants there occur in the resting nuclei chromatic bodies of the same number as the chromosomes during metaphase: these bodies enlarge directly into the prophase chromosomes. To certain other types of plant nuclei the views of Gregoire and his school apply. According to this idea the chromosomes as they pass to the poles become vacuolated, or alveolized, often forming in the resting nuclei reticulate bands which later are transformed into a spiral thread, the prophase chromosome of the next division. Of especial interest because of the very suggestive similarity of the method by which chromosomes are formed in *Fundulus*, is the view of Bonnevie resulting from the study of both plant and animal cells, *Allium* and *Ascaris*. She believes that there is genetic continuity of chromosomes although there is no *identity* between those of different mitoses: from the substance of each chromosome at the end of its life however, there arises endogenously the new prophase chromosome. The old chromosome is an earlier existing "endogen" for the foundation of the new. She regards the chromatic substance as the persistent continuing portion, while the achromatic substances between cell generations are lost. My observations on *Fundulus* bear out the main facts of this conclusion, although the details of the process differ.

It remains finally to inquire whether any significance attaches to the fact that the conditions described in this paper occur in tissues that are dividing rapidly. In all cases where persistent chromosomal structures are found the period of interkinesis is comparatively short; the question may be raised therefore whether in cells in which the interkinesis is of long duration the fusion of the vesicles is not more complete and the continuity perhaps lost. It seems to the writer that this is probably not the case, although the latter cells may require a more delicate technique and more close observation to discover that the vesicles are persistent entities. The fact that there is no resting period intervening between the maturation divisions and that the chromosomes in this case persist from one mitosis to the next in itself argues for the view of continuity elsewhere. We may look upon this discrete nature of these chromosomes as a manifestation of a condition that is general for cells and is merely emphasized in this particular case. A somewhat similar principle is that of a catalyzer, which is not able to initiate a reaction, but merely to hasten one that ordinarily takes place at a much slower rate. A second reply to the question of the significance of the above mentioned fact is that usually cells which divide very slowly are not of hereditary importance since those cells which bear the hereditary qualities usually divide with fair rapidity.

Summary.

The chromosomes in the eggs of *Fundulus* can be traced from the metaphase of one cell generation through all the stages of mitosis and interkinesis as continuous structures which give rise to the prophase chromosomes of the next mitosis. As they pass toward the poles in the anaphase they gradually loosen up, showing their constituent chromomeres, and finally form vesicles which come together in the telophase. Although they are closely applied to each other in the resting nucleus, they still maintain their unity of structure. When the nucleus begins the next division the new chromosomes are produced endogenously, each within the substance of one of the old vesicles which persists until the new chromosome is well formed. Thus there is clearly a continuity of substance from the old to the new metaphase chromosome. These observations establish clearly the genetic continuity of the chromosomes for this case and the evidence in general is so strong that the fact may be held as of wide application. The preceding account gives in detail the facts as observed in the mitoses of *Fundulus* eggs, a general summary of the occurrence of chromosomal vesicles and the relation of other cases to the present one, and finally a general statement of the present status of the theory of genetic continuity of chromosomes with the main points of evidence for it.

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EXPLANATION OF PLATE I.

These figures were all drawn with the aid of a camera lucida and Leitz two mm. objective with twelve compensating ocular (except figure sixteen as noted below). They are reproduced here at a magnification of a little more than eighteen hundred diameters.

FIG. 1. F♀ × F♂ normal; fifth cleavage division. Metaphase plate of which the chromosomes of two levels only are shown. Stage of maximum condensation of chromosomes.

FIG. 2. F♀ × C♂. Both radiated for three minutes before fertilization; second cleavage division. The beginning of chromosome division and first signs of separation of chromosomes are shown in this part of metaphase.

FIG. 3. F♀ × C♂; eggs radiated for fifteen minutes before fertilization; second cleavage division anaphase. The loosely formed chromosomes show increased size, the linin membrane and the chromosomes.

FIG. 4. F♂ × C♂; sperm radiated for fifteen minutes before fertilization; second cleavage division; chromosomes in similar condition to Fig. 3.

FIG. 5. F♀ × C♂; sperm radiated for three minutes before fertilization. The beginning of the vesiculation of the chromosomes. Liquid is being taken into the linin sac which in consequence swells up. A few of the chromosomes from both ends of the anaphase spindle are shown.

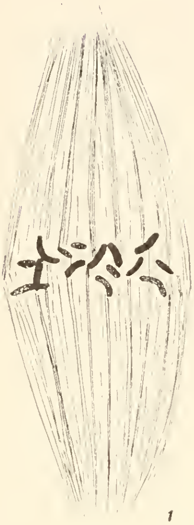
FIG. 6. F♀ × C♂; eggs radiated fifteen minutes; from one pole of anaphase of second cleavage. Same process carried farther.

FIG. 7. F♀ × C♂; both radiated three minutes; third cleavage division. A section through one pole of anaphase. All chromosomes are vesicles, showing no fusion, no condensation. The swelling process is still proceeding. The arrow points to the position of the sphere.

FIG. 8. F♀ × C♂; both radiated three minutes; fifth cleavage division, telophase. A somewhat oblique polar view. Nuclear division completed, but cytoplasmic not yet fully accomplished. Chromosomal vesicles further swollen, but quite distinct.

FIG. 9. Same material as Fig. 8. Telophase from one end of an incompletely divided fifth cleavage spindle. A so-called "reconstruction stage," with no sign of fusion of vesicles.

FIG. 10. Same material and stage. The aster at the right end is drawn in position from next section. The distinctness of the chromosomal vesicle at the side is particularly to be noted.



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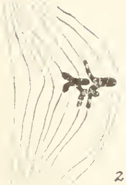

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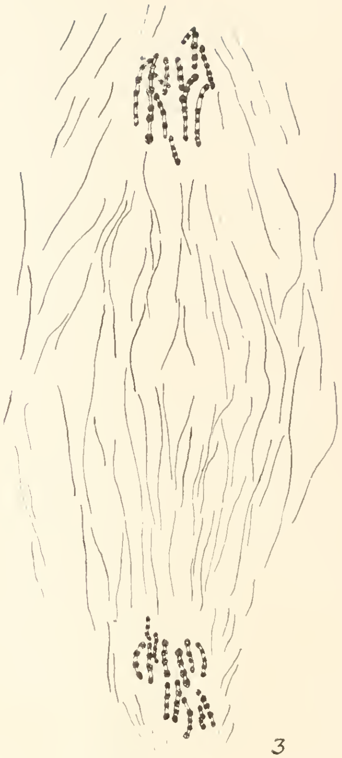

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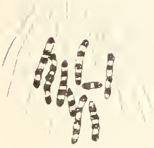

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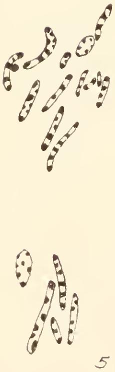

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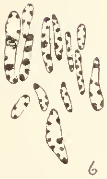

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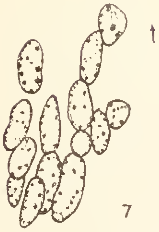

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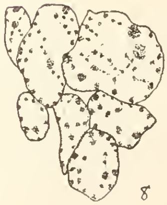

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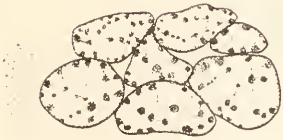

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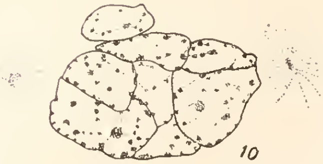


10

A. RICHARDS

EXPLANATION OF PLATE II.

FIG. 11. Same material and stage as preceding. Similar conditions shown. Rest of nucleus in next section.

FIG. 12. Same. It is to be noted that the division of the sphere has already taken place for the next mitosis.

FIG. 13. F♀ × C♂, normal material; condition of nucleus before the first cleavage division showing the distinct vesicles. Dotted line marks the area formerly occupied by this nucleus, shrinkage in preparation for ensuing mitosis having already occurred.

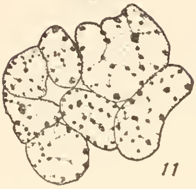
FIG. 14. F♀ × C♂; radiated for three minutes after fertilization. A nucleus from one-cell stage.

FIG. 15. F♀ × C♂; both radiated for three minutes before fertilization. Typical resting nucleus of second cleavage mitosis.

FIG. 16. F♀ × C♂; drawn with 6 objective and 4 ocular to show change in cell polarity. Blastula stage; upper cell is ectodermal; chromosomal vesicles shown in outline. Original axis was in line *AB*. With the shifting of the position of the spheres in telophase the axis of the upper cell came to line in line *CD* and that of lower in line *EF*.

FIG. 17. F♀ × C♂; eggs radiated for fifteen minutes before fertilization. Beginning of prophase of second division. Vesicles all distinct, showing a very definite polarity. Chromatin granules uniformly small and well distributed. This cannot be interpreted as merely a tangential section showing lobations, for the next section shows quite the same conditions.

FIG. 18. F♀ × F♂; from slide loaned by Miss Pinney as are Figs. 19, 20, 21 and 23. First cleavage similar to Fig. 17.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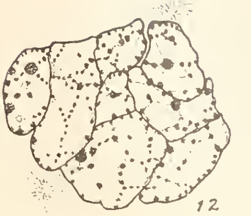
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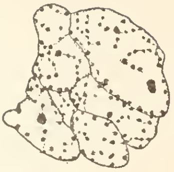
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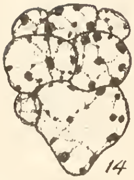
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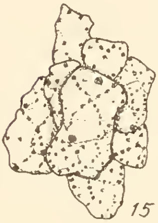
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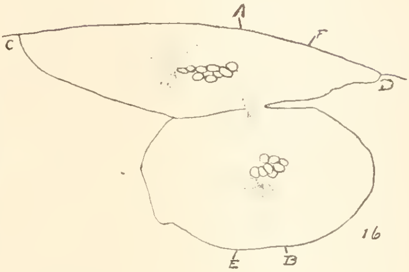

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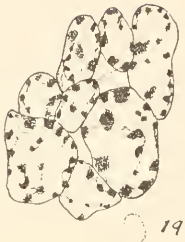
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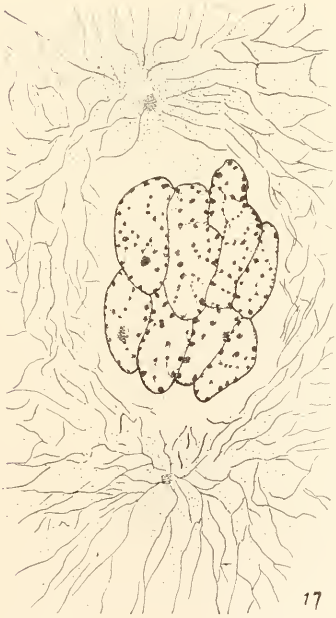
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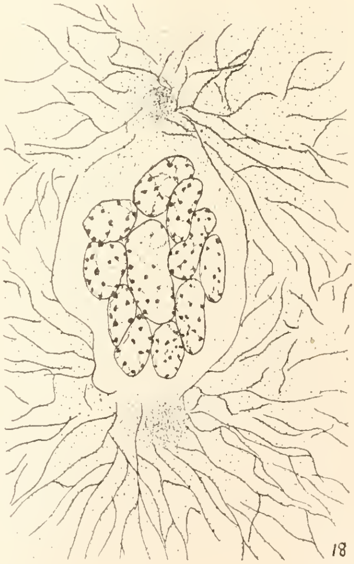
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EXPLANATION OF PLATE III.

FIG. 19. Pure *Fundulus* material. First step toward chromosome formation is shown in the condensation of chromatin granules on vesicle walls leaving the centers of the vesicles relatively empty.

FIG. 20. Pure *Fundulus* material. Further condensation and beginning of new chromosome as a row of granules, chromomeres, extending out into the center of the vesicle. Drawn as nearly as possible at one level.

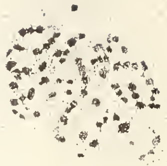
FIG. 21. All chromatin granules now formed into the new chromosome anlage. But the vesicular area can yet be distinguished. Only one level drawn.

FIG. 22. F♀ × F♂; radiated three minutes after fertilization. Prophase of third cleavage, showing distinctly old vesicular walls, and also new chromosomes already formed within. Certain of the vesicles show walls especially clearly. Only one level drawn.

FIG. 23. From slide belonging to Miss Pinney. Third cleavage. The only case observed showing the splitting of the chromosomes. The condition of spindle and asters suggest that this splitting may be precocious.

FIG. 24. F♀ × F♂; radiated three minutes after fertilization. Sixth cleavage prophase. oblique section. Traces only of the old vesicular walls are left while the astral rays are making their way into the chromosomes which are more condensed.

FIG. 25. F♀ × F♂; normal. Fourth cleavage, late prophase. As usual, only part of chromosomes are shown. Spindle formation completed and chromosomes are condensing and forming the equatorial plate.



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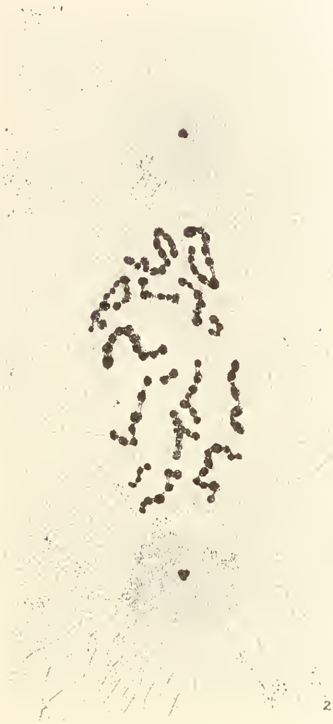

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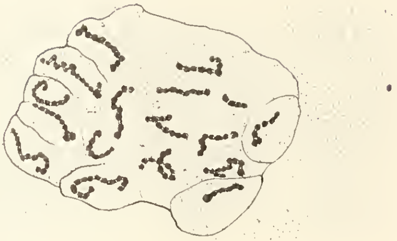

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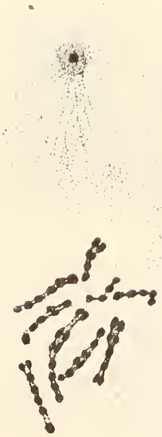

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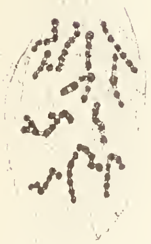

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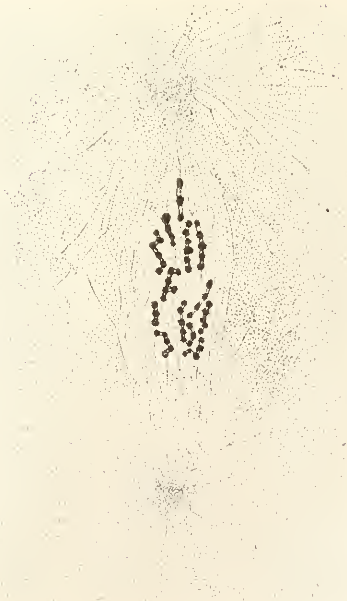

25

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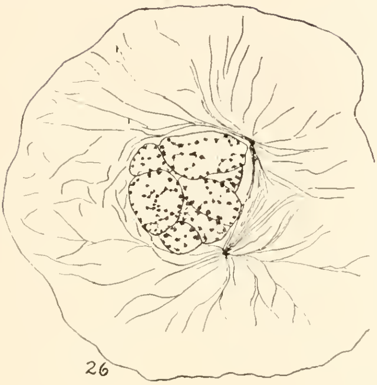
EXPLANATION OF PLATE IV.

FIGS. 26 to 29 are from a grastrulation stage of F♀ × C♂; sperm radiated fifteen minutes before fertilization, by which the sperm chromatin was killed so that it took no further part in development.

FIG. 26 shows the central spindle forming across one side of the nucleus.

FIG. 27 is a later stage showing the central spindle half dividing the nucleus. Not female and male parts, for the sperm's contribution of chromatin was killed as shown in Fig. 29, an anaphase in which all chromosomes are of the *Fundulus* type, and in Fig. 30, which is a cross-section of such a nucleus showing the groove through which the central spindle passes.



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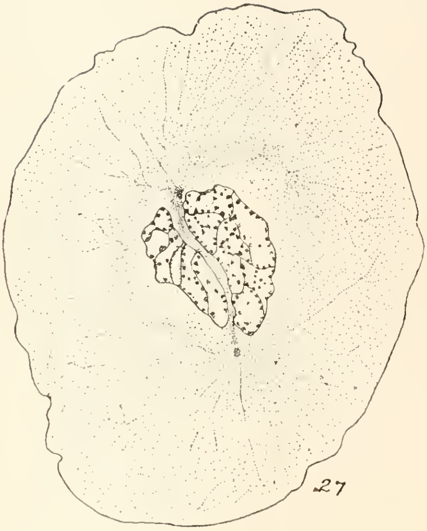

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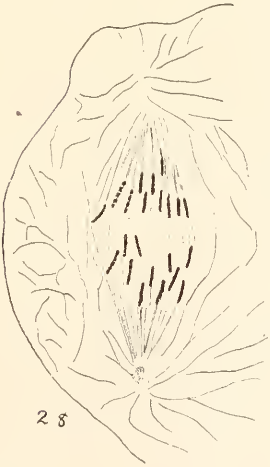

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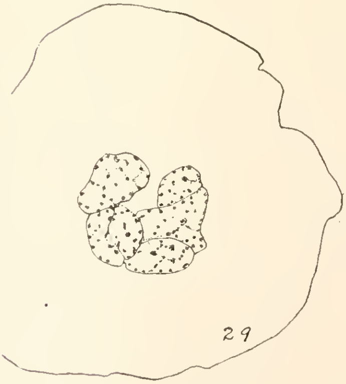

29

A. RICHARDS.

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BIOLOGICAL BULLETIN

STUDIES ON THE BIOLOGY OF PARACOPIDOSOMOPSIS.

I., DATA ON THE SEXES.[1]

J. T. PATTERSON.

I. INTRODUCTION.

According to Marchal ('04) and Silvestri ('06, '08) sex-determination in the polyembryonic chalcids is brought about in the same manner as in the bee. It is well known that the fertilized egg of the bee produces a female (queen or worker), while the unfertilized egg gives rise to a male (drone). Marchal and Silvestri believe that the fertilized egg of these parasitic hymenoptera produces a polyembryonic brood of females, while the unfertilized egg develops into a brood of males. This conclusion has been widely accepted, not only because it is in harmony with the Dzierzon theory of sex-determination in the bee, but also because several different investigators have observed that a polyembryonic brood usually does not contain individuals of both sexes. The occasional appearance of a mixed brood has been explained by the obvious and apparently logical assumption that a fertilized and an unfertilized egg had been deposited in a single egg of the host. The simultaneous development of two such eggs would naturally produce a mixed brood of parasites.

In a recent paper dealing with the development of the polyembryonic chalcid, *Copidosoma gelechiæ*, the writer ('15) called attention to the fact that it was difficult to explain the origin of certain mixed broods of this species on the assumption that two parasitic eggs carrying the opposite sex factors had been deposited in the egg of the host. It was suggested that of the nine complete, mixed broods listed, the origin of only five (broods 2, 3, 4, 6, 7, Table I.) could easily be explained in this obvious way. In each of the four remaining broods (broods 1, 5, 8, 9) the great preponderance of females seemed to indicate that such an explanation was not tenable. It was therefore suggested that these mixed broods could have arisen from a single fertilized egg, through a differential division of the two sex chromosomes at one of the early divisions of the egg. In that connection the following statement was made: "If *Copidosoma* conforms to the general scheme for sex-determination in insects, the females must have the 2 X chromosomes, and males the single X chromosome. Ordinarily, during the process of cleavage, all of the chromosomes in the fertilized egg divide equally, so that all of the nuclei entering into the formation of the embryos will carry the XX chromosomes, thus producing a brood of females. But if during the early development of the egg it should happen that the two X chromosomes in one or more cleavages should not divide but separate, one going to each pole of the spindle, each daughter nucleus would then receive a single X chromosome. If such nuclei later divide in the typical manner and give rise to embryos, such embryos would be males."[1]

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[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 130.

Different workers have from time to time offered a similar explanation to account for certain peculiarities in sex heredity, and especially to account for the origin of hermaphrodites and gynandromorphs. In doing so they have recognized the possibility that a qualitative division of the hereditary material for the sex factor may take place during cleavage. Their statements refer of course to the development of a monembryonic egg, in which the products of any somatic segregation must necessarily be distributed to different parts of the same embryo. In the case of a polyembryonic egg, a similar segregation, if occurring early enough in cleavage, would result in the production of two groups of embryos of different sexuality, and upon completing their development these two groups of embryos would produce a mixed brood.

It was this kind of evidence, although meager in amount,

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[1] *Loc. cit.*, p. 356.

that was discovered in studying the broods of *Copidosoma*. Only four out of a total of 162 broods gave evidence suggesting that a differential division might have occurred during the segmentation of the egg. It was therefore evident that if such differential divisions do occur in these parasitic insects, it must be studied in a species in which the polyembryonic brood is much larger than that of *Copidosoma*. For it is clear that the larger the brood the greater must be the number of cell divisions in that period of development at the close of which each blastomere becomes the progenitor of an embryo, and consequently. the greater the opportunity for a differential division to occur,

II. Data on Sexes of Paracopidosomopsis.

At Austin such a species is found in *Paracopidosomopsis floridanus* Ashmead, a form very similar to the European chalcid, *Litomastix truncatellus*, upon which Silvestri worked. This little parasite lays its egg in the egg of the common cabbage looper, *Autographra brassicæ*. This species is very favorable for study, and can be reared in the laboratory. Upon consuming the contents of the *Autographra* larva, the larval parasites pupate within the skin of the host, forming the typical mummified carcass. During the last two seasons about two hundred carcasses have been collected from the field. The silken cocoons were removed from these, after which each was placed in a vial, which was closed with a loose plug of cotton. Under these conditions the parasites readily emerge.

Table I.

Mixed Broods of *Copidosoma*.

Brood.	No. of Individuals.	Females.	Males.
1	162	153	9
2	172	92	80
3	207	126	81
4	216	176	40
5	235	223	12
6	241	161	80
7	300	235	65
8	304	292	12
9	337	316	21

One hundred and twenty-nine of these broods, taken at random, have been studied in detail, with the result that a number of interesting facts concerning the sexes have been brought to light. Of the hundred and twenty-nine broods, three are pure female broods, sixteen pure male, and one hundred and ten mixed. The three female broods have 1,089, 1,306, and 1,859 individuals, respectively; while the sixteen male broods run as follows: 385, 432, 525, 578, 675, 699, 988, 1,003, 1,124, 1,277, 1,285, 1,288, 1,333, 1,354, 1,492, 1,588. This is an average of 1,001 individuals per brood.

It is the mixed broods which are of special interest. The number of individuals in these broods varies from 545 to 2,028, with an average of 1,246 individuals per brood. The percentage of males varies from 72.07 to .06. In Table II. are listed sixty broods, in which the sex of each individual has been carefully determined. These are arranged in the order of per cent. of males, from the highest to the lowest. Fifty additional broods are listed in Table III., in which the total number of individuals is given, and the per cent. of males as determined by a study of five hundred individuals in each brood. This method of studying the sexes does not give the exact number of males and females in a brood, but it yields a very close approximation to the true per cent. of males.

These data raise a number of interesting points, only a few of which can be considered here. One of the most striking features is the scarcity of pure broods. Of all the broods studied, only about twelve per cent. are male broods, and less than three per cent. female broods. Another equally striking feature is the great preponderence of females in the mixed broods. In only seven of the one hundred and ten such broods is the number of males in excess of the number of females (broods 1, 2, 3, 4, 5, 6, 61, Tables II., III.). In the remaining one hundred and three cases the females are in the majority.

Reference to Tables II. and III. will show that in over 58 per cent. of the broods less than ten per cent. of the individuals in any given brood are males, while in 35 per cent. of the cases there is less than three per cent. of males. The most striking cases are those listed toward the bottom of the tables, especially the last six or seven broods in each table. In each of the last

TABLE II.

MIXED BROODS OF *Paracopidosomopsis*.

Brood.	No. of Individuals.	Females.	Males.	Per Cent. of Ma
1	974	272	702	72.07
2	1,289	400	889	68.97
3	1,237	482	755	61.02
4	636	294	342	53.78
5	828	385	443	53.50
6	545	272	273	50.09
7	1,162	595	565	48.71
8	848	529	319	37.62
9	1,512	1,009	503	33.27
10	1,847	1,287	560	30.32
11	839	586	253	30.15
12	943	701	242	25.66
13	998	746	252	25.25
14	1,706	1,353	353	20.69
15	628	509	119	18.95
16	662	542	120	18.12
17	1,022	878	144	14.09
18	1,364	1,195	169	12.39
19	1,797	1,584	213	11.85
20	1,041	923	118	11.35
21	1,477	1,357	120	8.12
22	1,115	1,033	82	7.35
23	1,307	1,211	96	7.35
24	1,303	1,199	104	7.21
25	1,196	1,122	74	6.19
26	803	754	49	6.10
27	1,432	1,352	80	5.59
28	868	822	46	5.30
29	1,423	1,349	74	5.20
30	1,050	996	54	5.14
31	1,883	1,791	92	4.89
32	1,259	1,203	56	4.45
33	1,457	1,394	63	4.32
34	855	828	27	3.16
35	1,046	1,015	31	2.96
36	1,124	1,091	33	2.94
37	1,000	972	28	2.80
38	757	739	18	2.38
39	847	827	20	2.36
40	1,301	1,271	30	2.31
41	1,209	1,183	26	2.15
42	1,260	1,233	27	2.14
43	1,682	1,647	35	2.08
44	1,592	1,559	33	2.07
45	1,258	1,232	26	2.07
46	692	679	13	1.88
47	1,220	1,200	20	1.64
48	1,115	1,097	18	1.61
49	1,036	1,021	15	1.46
50	1,798	1,774	24	1.35
51	1,075	1,061	14	1.30
52	1,475	1,456	19	1.29
53	1,349	1,336	13	.96
54	2,003	1,986	17	.85
55	1,017	1,010	7	.69

TABLE II.—*Continued.*

MIXED BROODS OF *Paracopidosomopsis.*

Brood.	No. of Individuals.	Females.	Males.	Per Cent of Males.
56	1,165	1,159	6	.52
57	1,143	1,141	2	.18
58	1,493	1,491	2	.13
59	912	911	1	.12
60	1,550	1,549	1	.06

two broods of Table II. only a single male was present. *In contrast with this condition, broods with a large number of males and a few females have so far not been found.* As stated above, in only seven cases out of a hundred and ten are males in excess of females, and even in these the smallest number of females is almost as much as 28 per cent. of the entire brood (brood 1). The fact that females are so frequently in excess of males in the broods is one of the most significant points brought to light in the study of these parasites.

TABLE III.

MIXED BROODS OF *Paracopidosomopsis.*

Per Cent. of Males Based on a Study of 500 Individuals.

Brood.	No. of Individuals.	Per Cent of Males.	Brood.	No. of Individuals.	Per Cent. of Males.
61	2,028	67.20	86	701	11.00
62	1,043	47.50	87	1,403	9.20
63	1,149	44.00	88	1,602	8.20
64	1,540	43.90	89	1,606	8.09
65	1,463	42.80	90	1,228	8.00
66	1,134	37.00	91	1,614	7.40
67	1,397	31.60	92	1,675	7.40
68	940	31.20	93	1,258	6.50
69	1,123	29.60	94	1,139	6.00
70	996	29.00	95	841	5.20
71	1,045	26.00	96	1,111	4.40
72	1,467	24.80	97	1,665	3.40
73	1,822	24.00	98	1,351	2.80
74	1,233	22.60	99	1,633	2.80
75	1,685	21.80	100	1,857	2.40
76	1,095	19.60	101	794	2.20
77	1,745	19.00	102	1,306	2.20
78	1,201	18.00	103	1,092	2.00
79	1,301	16.00	104	980	2.00
80	841	14.60	105	1,005	1.80
81	1,410	14.60	106	1,334	1.20
82	1,605	14.20	107	1,550	1.20
83	867	13.60	108	1,560	1.00
84	1,347	12.20	109	1,469	.60
85	1,167	11.80	110	1,227	.60

III. Suggested Explanations of Mixed Broods.

On the basis of the data here presented, how is one to explain the origin of mixed broods in *Paracopidosomopsis?* There are at least two possible explanations that may be suggested: (1) that a fertilized and an unfertilized egg had been deposited in the egg of the host; (2) that a fertilized egg had given rise to males as well as to females.

One finds no difficulty in offering the first of these suggested explanations to account for some of the mixed broods, especially for the larger broods in which males and females are found in about equal numbers. But if the same explanation is to be applied to all mixed broods, it would be necessary to assume that in the majority of cases the development of the unfertilized egg is interfered with by the presence of the developing fertilized egg. In the light of Lillie's ('16) recent discovery concerning the origin of the "free martin" in cattle, this assumption would not seem to be untenable. In the case of these insects it is conceivable that a fertilized egg might generate "hormones" which would inhibit the development of an unfertilized egg in such a way that only a few of the male embryos reach maturity.

One difficulty which at first seemed to stand in the way of the application of the hormone theory is the fact that the vast majority (about 85 per cent.) of all *Paracopidosomopsis* broods are mixed. It did not seem possible that eighty-five per cent· of the moth eggs, widely scattered as they are in the cabbage patch, would each be visited and parasitized by a fertilized and an unfertilized female insect. This raised the question as to whether the female lays more than a single egg at each oviposition, and if so, whether the fertilized female has the power to lay both kinds of eggs, as is the case of the queen bee. Silvestri states that the female of *Litomastix truncatellus* lays but a single egg at each oviposition. We have studied this question in *Paracopidosomopsis* and find that the female, whether fertilized or not, frequently deposits two eggs at one oviposition. In one experiment, a single unfertilized female made seventeen successive ovipositions in as many eggs. The time of oviposition varied from one minute and thirty seconds to three minutes, with an average of two minutes for each egg. An examination of these parasitized eggs in carefully teased preparations showed that the parasite had laid two eggs in eleven cases, and a single egg in six cases. The same experiment performed with fertilized females shows the same result. In a total of forty-two ovipositions made by three unfertilized and sixteen fertilized females, two eggs were laid in twenty-eight cases and one egg in fourteen cases.

While these observations show conclusively that two eggs are frequently deposited simultaneously by the insect, yet they do not settle the question as to whether the fertilized female can lay both kinds of eggs. However, a study of smear preparations and sections shows that in many cases both eggs are fertilized. For this, as well as for other reasons, the writer is of the opinion that any suggested explanation involving the idea that an unfertilized and a fertilized egg is laid in the egg of the host is inadequate to account for all of the mixed broods in *Paracopidosomopsis*.

This brings us to a consideration of the second suggestion, namely, that a fertilized egg may give rise to males and females. This result could be attained by the abnormal behavior of the sex chromosomes in one or more of the cleavage divisions. There are several different ways in which an abnormal behavior of the sex chromosomes might occur. Some of these possibilities are too improbable to receive consideration, but there are three or four that we shall mention. First, by *somatic disjunction*,[1] by which is meant the separation instead of division of the two sex chromosomes in a cleavage mitosis. Second, by *somatic non-disjunction*, by which is meant the failure of the two daughter chromosomes of one of the X's to separate after the division of both sex chromosomes had occurred. This would result in one daughter cell receiving three sex chromosomes, the other a single X chromosome. A third possibility is that only one X chromosome might divide, resulting in the formation of one cell with two X's and another with only one X. Finally, we may mention a fourth possibility, namely, that a division might occur in which all the sex chromosomes, whether divided or not, pass to one pole of the spindle. In this event one of the daughter cells would contain no sex chromosome. Let us consider further each of these possibilities.

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[1] This term is not entirely exact, because of the implication that the separating chromosomes had previously been united, which of course is not the case.

The mechanism of a disjunctional division was explained in connection with the study of *Copidosoma*, from which a quotation is given in the first part of this paper. It remains therefore to point out what would happen should such a differential division occur. If a disjunctional division should occur during cleavage, the percentage of males to females would be determined by the time at which the differential division occurs. For example, if disjunction took place at the first cleavage of the fertilized egg, each of the two daughter cells would receive a single X chromosome, and should no further disjunctions occur in subsequent divisions, a pure male brood would be produced. If on the same basis the disjunction did not occur until the second division, only fifty per cent. of the brood would be males; if at the third, twenty-five per cent.; if at the fourth, twelve and one half per cent., and so on decreasingly until the point is reached at which each cell becomes the progenitor of a single embryo. However, in any random lot of mixed broods these expected classes, based on per cent. of males would not be realized, because there are certain factors in development which would tend to modify the expected percentage of males in any given brood. Thus not all of the cells divide simultaneously in the early cleavages of the egg of these parasites. Some cells may fail to develop altogether. More than one disjunctional division may occur in the egg. Finally, some cells develop into the so-called assexual larvæ, which never reach maturity. In a random lot of broods we should therefore expect to find a continuous series, such as is shown in the tables.

Of the several possibilities suggested that of somatic non-disjunction would seem to be the most plausible. First, because it is in harmony with the known facts of cytology on cleavage mitosis, in that all of the chromosomes are supposed to divide; and second, because it is in accord with Bridges' ('16) work on non-disjunction in *Drosophila*. This investigator has recently suggested the possibility of somatic non-disjunction to account for gynandromorphs and mosaics in a monembryonic egg, such as that of *Drosophila*. He says: "If the same sort of primary non-disjunction which has been assumed to give rise to XX′X′ cells in an XX female, should take place at a cleavage stage, gynandromorphs and mosaics would result. One might expect at an early cleavage division, particularly the first, a relatively large number of X–XX′X′ divisions, for the greatly condensed chromosomes introduced by the sperm do not for some time attain the state or the appearance of those of the egg nucleus. If the paternal X of a female were slower than the maternal in preparing for division, it might lag upon the spindle so that both daughter X's would become included in the same cell. The portion of the fly which comes from the X cell should be male and should show the sex-linked characters of the mother. Such a process may be the explanation of the large number of lateral gynandromorphs of *Drosophila*. When the X–XX′X′ division occurs at a later cleavage stage we may have mosaics, as for example, a red female with a patch of white facets in the eye."[1]

In non-disjunction the per cent. of males in any brood would be determined, as in disjunction, by the time at which the division takes place. However, there would be this difference, that should the first cleavage be non-disjunctional, the expected percentage of males would be fifty instead of one hundred; should the second cleavage be non-disjunctional, twenty-five per cent. would be males instead of fifty, and so on, decreasingly throughout the series of cleavage divisions.

The third possibility mentioned above is in reality only a modified form of non-disjunction, and the percentage of males would be determined in accordance with the same law.

The fourth possibility suggested above is of interest because such a division would result in the production of blastomeres devoid of sex chromosomes. Cells which contain no X chromosomes may be responsible for the so-called asexual larvæ that develop from the egg of this species. We shall consider this point further in connection with a discussion of these peculiar larvæ.

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[1] *Loc. cit.*, p. 136.

IV. Experimental and Cytological Studies.

If any one of these forms of abnormal behavior of sex chromosomes does occur in the cleavage divisions of *Paracopidosomopsis*, it should be possible to detect it by experimental and cytological studies. We have undertaken both lines of investigation, and already sufficient progress has been made to clear up certain points.

The experimental work has been greatly interfered with by the ravages of the so-called wilt disease, to which the *Autographra* larvæ are extremely susceptible under laboratory conditions. In all of the experimental work the precaution has been taken to protect the moth eggs from being parasitized before beginning any experiment with them. This has been done by having the moth lay its eggs under a bell-jar from which all parasites were scrupulously excluded. Furthermore, the parasitized eggs and the young larvæ developing from them have been similarly protected. In all experiments the female parasite has been permitted to make but one oviposition in each moth egg.

The first set of experiments were undertaken with a view to determine whether the fertilized female parasite could lay eggs which produce males and females. In one experiment 23 moth eggs were parasitized by fertilized females. Sixteen larvæ hatched three days later. Of these two reached full growth. One of the two larvæ was not parasitized, and formed a pupa from which a moth later emerged. The other was transformed into a typical mummified carcass, from which a brood of parasites in due time emerged. The brood contained 2,096 individuals, of which 1,889 were females and 207 males. While these results are meager, nevertheless they indicate clearly that a fertilized female is capable of laying eggs from which individuals of both sexes develop. In this instance it is not possible to tell whether the mixed brood came from one egg or from two eggs, since there was no way of determining the number of eggs deposited by the parasite in the egg of the host at the time she made the oviposition.

In another set of experiments unfertilized females were used. In one experiment eighty moth eggs were parasitized, each by one oviposition by an unfertilized female. Sixty-six larvæ hatched. All but twenty-two of these larvæ were fixed at various stages of development, in order to secure material for the study of the unfertilized parasitic egg. Three of the twenty-two caterpillars eventually reach full or nearly full growth. Two of these showed signs of disease, and were killed, the parasitic larvæ being removed and fixed for cytological study. A study of sections of the larvæ from both caterpillars fail to show anything but male larvæ present. The third caterpillar formed a carcass, from which 1,842 males emerged. To this must be added 100 more individuals, which were removed from the carcass in the pupa stage and fixed for cytological purposes. The sections show that all the pupa are males. These results indicate that the unfertilized egg of *Paracopidosomopsis* produces males only.

A study of the spermatogenesis as revealed in these pupæ shows that males developed by parthenogenesis carry the haploid number of chromosomes, while a study of maturation of the eggs and fertilization shows that females, as would be expected, carry the diploid number. With these facts established, it will be possible to determine whether two types of males are produced, as may be shown by the following consideration. If *Paracopidosomopsis* conforms to the general scheme of sex determination in insects, the chromosomal formula for the fertilized egg may be represented by $N + 2X$, in which N represents the non-sex chromosomes, and that for the unfertilized egg by $\frac{N}{2} + X$. Now, should males also arise from the fertilized egg, their formula must be $N + X$. The presence of the double number of non-sex chromosomes in such individuals should not affect their sexuality, because in all forms in which parthenogenesis is absent, males are represented by this or a slightly modified formula. Modern genetic studies have shown that maleness or femaleness is determined by the number of X chromosomes present in the egg, and not by the number of non-sex chromosomes. Even in forms which have the Y chromosome, the presence or absence of the Y does not seem to affect the sex of the individuals.

V. The Asexual Larvæ.

Asexual larvæ were first described by Silvestri for *Litomastix*. According to this investigator, the egg of *Litomastix* produces a thousand or more sexual larvæ and a variable number of asexual larvæ, which are characterized by the absence of certain organs, and especially by the absence of the reproductive system. These larvæ die without undergoing metamorphosis. Exactly similar larvæ develop from the egg of *Paracopidosomopsis*. The details of their development have not as yet been studied, so that I cannot say whether they appear in every egg or not. These curious larvæ develop both in the fertilized and in the unfertilized egg, even though the host egg be rigidly protected from all other parasites. This shows that the asexual larvæ do not come from the egg of another parasitic species, as has been suggested as an explanation for their appearance among the larvæ of *Litomastix* (Wheeler, '10). An account of their development will be made the subject of a subsequent paper. Here, we are concerned with the question of the underlying cause of their production.

Silvestri suggests that these asexual larvæ may owe their asexuality to the absence of germ cells. It will be recalled that he described for the polyembryonic egg a so-called nucleolus (nucleolo), which lies at the larger or posterior end of the elongated, bottle-shaped egg. In the early cleavage stages of *Litomastix* the nucleolus becomes included in a single blastomere, resulting in the retardation of the subsequent divisions of this cell. The nucleolus later breaks down and becomes scattered evenly throughout the cytoplasm. Silvestri was unable to follow the history of this particular cell beyond two divisions, but he believes that it gives rise to all the germ cells of the sexual embryos. His conclusion has been strengthened by a subsequent study of the eggs of two monembryonic parasites. The nucleolus in the egg of these species becomes distributed to the germ cells alone, and thus serves as a "keimbahn-determinant" (Hegner).

According to Silvestri's suggestion the asexual larvæ of *Litomastix* arise from the polygerm, but fail to receive germ cells or descendants of the single blastomere which inherited the nucleolus. It may be that this suggestion of Silvestri offers the correct explanation of the origin of the asexual larvæ, but there are certain obstacles which stand in the way of its full acceptance. In the first place, it is difficult to conceive of any mechanism in the complex polygerm by which predestined germ cells could be nicely and exactly distributed to each of the several hundred sexual embryos which develop from the eggs of *Litomastix* and *Paracopidosomopsis*. In the second place, it is a well-known fact that in insects secondary sexual characters and also certain primary sexual characters, such as the organs of copulation and oviposition, are not dependent upon the presence of the gonads for their development. This conclusion is based upon trustworthy evidences obtained from castration experiments on young larval stages. Furthermore, in the light of modern genetic evidence, I should be inclined to believe that the asexual larvæ are sexless not because they have failed to inherit predestined germ cells, but because of their failure to inherit X chromosomes. If X-free blastomeres are formed during cleavage, such cells might become the progenitors of sexless larvæ, which in a way could be compared to the non-viable, OY zygote of *Drosophila*.

VI. Data on Sexes of Other Polyembryonic Insects.

With the exception of *Copidosoma gelechiæ* and *Paracopidosomopsis*, exact data on the sexes of polyembryonic insects are very few. The first observations recorded are those of Bugnion ('91), who studied twenty-one cases of *Ageniaspis* (*Encyrtus*) *fuscicollis* and found the following conditions: nine pure female broods; five pure male broods; three broods with males and females in about equal numbers; three broods with large majority of males; one brood with large majority of females. In commenting upon these data, Marchal ('04) points out that it is difficult to explain the four cases in which the large majority of individuals belong to one or the other sex. He believes, however, that such broods arise from two or more eggs. He further believes that should two eggs, one fertilized and the other not, be laid in the egg at slightly different times, the one deposited first might gain the upper hand in the matter of food, and thus prevent all but a few of the individuals arising from the second egg from reaching maturity.

Marchal examined sixteen broods of *Polygnotus minutus*, and found eight female broods, six male, and two mixed, one with three females and one male, the other with three females and three males.

The only other investigator to record data on the sexes of polyembryonic parasites is Silvestri. In one hundred and sixty broods of *Litomastix* he found sixty-three female, ninety-two male, and five mixed broods. He does not record the number of individuals for any of these broods.

I should like to point out in conclusion that there is very great need for exact data on the sexes of polyembryonic insects.

AUSTIN, TEXAS,
January 17, 1917.

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ON THE PRODUCTION OF MONSTERS BY HYBRIDIZATION.

H. H. NEWMAN.

Of late there have appeared several papers that deal with the production of monsters in teleosts, especially in the common mud-minnow, *Fundulus heteroclitus*. The papers of Kellicott ('16) and Werber ('15 and '16) have been especially interesting because they deal with the same type of monster that I have been studying incidentally for more than ten years and that I have described and discussed in several different papers dealing with development and heredity in fish hybrids.

In 1908 I published the results of intercrossing *Fundulus heteroclitus* and *F. majalis* (Newman, '08). In the cross *F. heteroclitus* × *F. majalis* I called attention to the various types of monsters, noting especially those types in which the head and eyes developed and the body was relatively inhibited, and those in which head development was inhibited and body was relatively normal.

The wide range of variability in the results of crossing two individuals of different species was emphasized. A long list of conditions was given ranging from extreme subnormal eggs that failed to cleave, up to markedly supernormal individuals that are hardier and better equipped to live than individuals of either pure strain. In subsequent papers (Newman, '12 and '14) I called further attention to the graded series of teratomata resulting from one set of eggs fertilized by sperm from a single male.

In general it was shown that in hybrids between closely allied species (homogenic hybrids) the rate of development of a considerable percentage of individuals was accelerated, but that in all heterogenic hybrids there is a more or less marked retardation of developmental rate from the earliest cleavage stages on. Even in the case of homogenic hybrids, the retarded individuals are always abnormal.

During the summers of 1914 and 1916 a series of experiments was performed in order to test the theory that retardation is always correlated with abnormal development. In hybrid experiments in which *Fundulus heteroclitus* eggs are fertilized by the sperm of the mackerel there is noticeable in comparatively early cleavage stages a marked difference in number and uniformity of blastomeres in different eggs of the same batch. The different types of eggs were divided into three lots as follows:

A. Those that were most advanced and exhibited the largest number and greatest regularity of cells.

B. Those in which the cells were fewer and less uniform.

C. Those in which the cells were relatively fewest and of various sizes.

The development of these three lots was watched from day to day with the result that all of those in A reached a fairly advanced condition before abnormalities appeared and abnormalities concerned chiefly the eyes and heart; those in B showed all sorts of monsters including cyclopeans, eyeless types, and those with reduced bodies; those in C produced solely apical parts without bodies, amorphous masses of tissue or at best isolated organs such as eyes and hearts.

The converse of this experiment was tried using the eggs of *F. heteroclitus* and the sperm of the closely related *F. diaphanus*. In this experiment there was very little acceleration noticeable during the cleavage stages, but during gastrulation it was a simple matter to separate out three classes A, B, and C as follows:

A. Those in which the germ ring was distinctly more than half way round the yolk.

B. Those in which the germ ring was approximately half way round the yolk. These were about like the control.

C. Those in which the germ ring was distinctly less than half way round the yolk.

The eggs in lot A nearly all hatched on the average distinctly earlier than the control (pure *F. heteroclitus*), and were distinctly more active, grew faster and lived longer than the control. Those in lot B showed a wide range of variability, some hatching as early as the control, others hatching later, and still others failing to hatch. Various common types of abnormality occurred but none of the most extreme types were noted. Those in lot C showed the most pronounced abnormalities. Only a few hatched and these were rather feeble. The remainder were monsters belonging to a wide range of types, many showing merely some defect in the circulation (string-hearts, enlarged pericardia, no blood, etc.), others being defective in the head parts, especially eyes, and others being defective chiefly in the posterior parts.

These two experiments were repeated many times and gave substantially identical results each time. There can be no question therefore that *there is a very close correlation between the rate of development and the degree of normality of the embryos.*

When in homogenic crosses a slight increase in rate of development is instituted the result is a supernormal F_1 hybrid type, in which the difference is largely physiological, consisting of greater activity and greater viability. When in the heterogenic crosses there results a more or less pronounced retardation in developmental rate (sometimes clearly seen in the early cleavage stages) a subnormal type or monster is the result, which differs from the normal not only physiologically in being non-viable but morphologically in being defective in one or more parts.

Both supernormal and subnormal types appear then to be correlated with an alteration in the normal rate of development.

It is quite evident from numerous experiments (Newman, '15) that the degree of retardation resulting from varous heterogenic hybridizations is not a factor of the distance of the cross, for some species of the same genus cross with poor success, and some species of different orders of teleosts cross so as to produce swimming larvae. This argues against the theory that foreign sperm introduces toxins, since the protoplasm of distantly related forms ought to be more toxic than that of near relatives.

About all we can say then is that *in introducing a foreign sperm into an egg we either accelerate or retard the developmental rate of the egg.* In proportion as the rate is accelerated we get a supernormal result, and in proportion as the rate is retarded, a subnormal result.

That there is nothing specific about the effect of foreign sperm upon the developmental rate of the egg is further shown by the fact that the same series of subnormal types (monsters) are found in a great variety of different crosses, and also by the fact that these same abnormal types may be produced by purely physical or by chemical agents.

Other Methods of Producing Monsters.

As long ago as 1907, Stockard, by the use of solutions of magnesium chlorid, was able to produce, among other abnormal conditions, a considerable percentage of cyclopic and allied ophthalmic terata. Although little was said about abnormal conditions other than those associated with the particular terata mentioned, enough data was given to show that many if not all of the other well-known conditions occurred.

In 1909 and 1910 Stockard contributed further papers concerning the development of teratomata, showing that the same results could be obtained with alcohol and other anæsthetics. He called particular attention to ophthalmic terata, making it clear that the eye anlages appear to be especially susceptible to anæsthetics, and explained cyclopia and similar conditions as "the result of an anæsthetic action during the early developmental stages." Later, when McClendon ('12) obtained a similar series of eye defects by the use of substances that are non-anæsthetic in action, Stockard took the more general view that deleterious chemical substances merely lower the vitality of certain sensitive anlages and cause arrested development of important structures. Stockard implies that the results are primarily due to a lowering of developmental rate. The paired eyes for example are believed to arise from an unpaired median anlage which normally undergoes a sort of twinning process producing two eyes. Under the influence of deleterious chemicals "the median anlage does not widen or spread laterally but is arrested in its primary median condition." Hence the unpaired cyclopean eye develops.

Recently Werber (1915, 1916) in three papers and especially in his latest one (1916) re-attacked the problem from a somewhat novel point of view. Starting in with the assumption that the development of monsters in mammals is caused by "pathologic parental metabolism" he decided to test his hypotheses with fish embryos. He consequently subjected the embryo of the fish *Fundulus* to solutions of various metabolic by-products, characteristic of normal and pathologic mammals, such as urea, butyric acid, lactic acid, acetone, sodium glycocholate, and ammonium hydroxide. Conclusive results were obtained only with butyric acid and acetone, especially the latter. The paper is especially valuable for its comprehensive record of terata, their classification, and the numerous figures of monsters of every sort. All of the results are similar to those obtained in hybridization experiments, as is evidenced by the fact that the illustrations would serve as well for a paper on hybrid teratomata as for the paper in which they appear. All of Stockard's teratomata are also duplicated exactly.

It is especially to be noted that there are two markedly different types of monsters: (*a*) those in which the head parts alone are inhibited, and especially the eyes and heart, and (*b*) those in which the head parts develop fairly normally and the posterior parts are inhibited. Extremes of (*a*) are seen when only the eyes or heart are effected, and of (*b*) when eyes or heart develop alone to the exclusion of other parts of the body. These results appear at first sight quite paradoxical, but the explanation will be clear from what follows.

Werber appears to realize the importance of Child's theory of the "axial gradient" in organisms as an aid in understanding the morphogenesis of monsters. He points out that the anterior end, or "apical end" is most susceptible to agents that inhibit development, and hence we have a logical basis for the frequency of ophthalmic terata. He sees in Child's work however no explanation of the occurrence of heads with reduced body and of isolated eyes, hearts, etc.

Werber's idea of the morphogenesis of monsters differs somewhat from that of Stockard in being strongly morphological. According to him any chemical substance, whether anæsthetic, toxic, or what not, so long as it is injurious to life, checks the process of cell metabolism and cell division and causes the affected cells to *disintegrate*. This "*differential blastolysis*" accounts to Werber's mind for the suppression of certain anlages, especially of those at the apical end of the embryo; but it fails to account for the development of isolated eyes and hearts in other embryos of the same series, which are treated exactly as those were in which the eyes were suppressed or the heart failed to develop.

It will be seen that both Stockard and Werber believe that abnormal development is the result of toxic or deleterious substances introduced into the egg from without. It has been shown however that identical results can be obtained by purely physical methods.

In a very recent paper Kellicott ('16) has reported the *production* of every known type of teratomata in *Fundulus* by merely subjecting very young embryos to low temperatures. Eggs placed in the refrigerator a few minutes after insemination very nearly stopped development and some apparently underwent regressive changes. When returned to normal temperatures these eggs developed nearly every type of abnormality. The most frequent terata were those of the eyes and of the heart. A list of terata would be a mere repetition of those obtained by other methods.

By way of a theory of the genesis of monsters Kellicott proposes what he calls a "disorganizational hypothesis," namely "that the cause of abnormal and monstrous development, is to be found in a disturbance of the normal organization of the ovum, as expressed by the unusual character and distributions of the differentiated materials of the egg protoplasm." It should be noted in connection with these low temperature experiments that the first result is a very pronounced lowering of developmental rate, amounting in some cases almost to a cessation of development, accompanied by certain regressive changes. There is also in all cases a permanent retardation even after the embryos are returned to normal temperatures. It seems then that *the primary effect of low temperature is the lowering of metabolic rate.*

It will now readily be seen that the whole series of teratomata in fish eggs may be produced in three entirely different ways, the results being the same in each:

1. By the use of chemicals whose action is such as to lower the rate of metabolism.

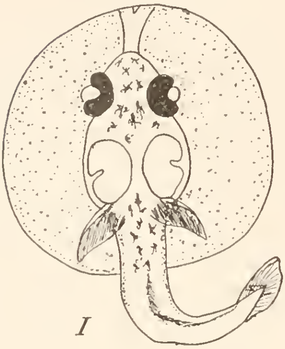
2. By means of low temperatures, which ~~e~~affect~~s~~ directly the rate of metabolism.

3. By means of heterogenic hybridization, which always effects a lowering in the rate of development.

All monsters then seem to be the result of a lowering of developmental rate.

Let us now return to a consideration of hybrid monsters and ask ourselves the question: *how does the foreign sperm lower the rate of development?* Various views are held as to the way in which the foreign sperm retards development.

Moenkhaus holds that the foreign sperm exercises a poisonous or toxic effect in proportion to the distance of the cross. Loeb holds that the results are due to the inability of the hybrid to digest the yolk with sufficient rapidity. I have expressed the view that there is an incompatibility between the nuclear elements of the two different species that results in abnormal chromosome arrangements and irregular distributions of the latter to various cells. Undoubtedly also the cleavage mechanism works less perfectly when chromosomes of very different size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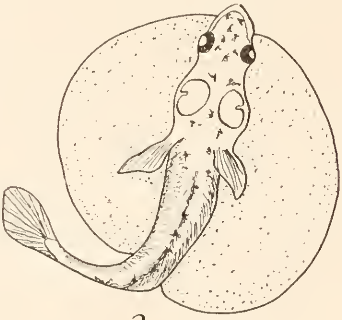
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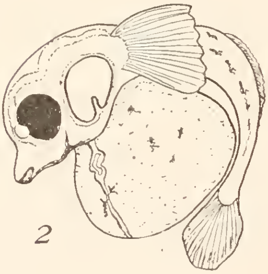
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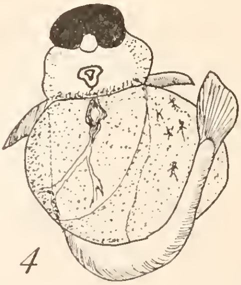
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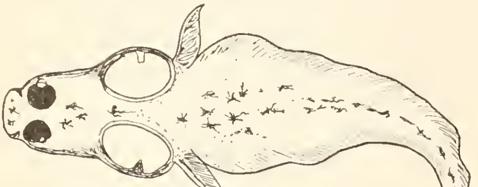
and form are mixed together in a single cell, than when they are all of the same kind. Whatever the primary cause of abnormal development may be, the most obvious early effect is a lowering of developmental rate and it is on this point that the similarity exists among all of the experimental results heretofore discussed.

Since we are dealing primarily with rates of metabolism, we shall doubtless find, as Werber has suggested, that the morphogenesis of monsters in teleosts is simply a special case of form regulation similar to those recently described and discussed by Child ('15 and '16) for sea-urchins, in which he demonstrates the effectiveness of the axial metabolic gradients as dynamic factors in the development of various types of monsters. He has been able to "control and modify development by means of the differential action of external factors on different regions of these gradients."



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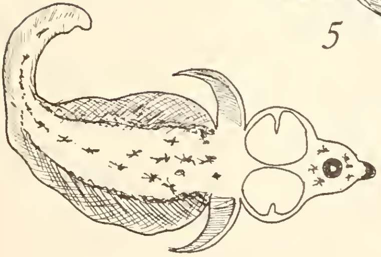

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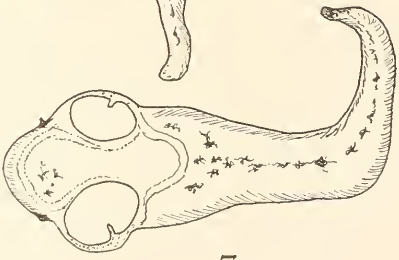

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It is my belief that an application of the principles enunciated by Child serve to rationalize the results of heterogenic hybridization as well as those produced by chemicals and by low temperatures, and since Werber merely suggested the possibility of explaining ophthalmic anomalies by this theory and failed to apply it in any far-reaching way to the other various types of monster he was dealing with, it seems worth while to give the theory a thorough trial in this field.

THE AXIAL GRADIENT THEORY AND THE MORPHOGENESIS OF MONSTERS RESULTING FROM HETEROGENIC HYBRIDIZATION.

The axial gradient in vertebrates is essentially a quantitative one depending on the rate of metabolic activity. The rate along the apico-basal axis is highest at the anterior or apical end and lowest at the posterior or basal end. Paralleling the axial metabolic gradient there exists a susceptibility gradient, in which the apical end is most susceptible to agents that lower the vitality or retard the rate of metabolism. Similarly the median dorsal region is the most susceptible point of the bilateral or mesiolateral axis and the lateral part the least susceptible. Certain points that are both apical and median will be the most susceptible of the whole system. The eyes are such structures developed from a primitive mesio-dorsal-apical anlage, and are therefore the most susceptible of all parts of the body.

In animals of the high grade of complexity exhibited by vertebrates there are to be distinguished several semi-independent axiate systems. Undoubtedly the central nervous system and its derivatives constitutes an axiate system by itself. Similarly the circulatory system is axiate with the heart the apical point and the veins and arteries basal. The skeleton with the fin systems are also semi-independent axiate systems, with the anterior parts more susceptible than the basal ones. This is brought out in recovery cases in which the pectoral fins recover while the pelvic do not.

Whenever young fish embryos are subjected to any of the types of inhibiting or retarding agents that have been previously discussed we are able to classify the resulting abnormal forms into categories (modifying somewhat the usage of Child): (*a*) Forms in which there has occurred an inhibition of anterior and of median dorsal regions; (*b*) Forms in which these regions have become acclimated or have recovered from an initial inhibition. Curiously enough in chemical experiments those parts that are most susceptible to inhibiting agents of higher concentrations are able most completely to acclimate themselves in lower concentrations or to recover when the inhibiting agent is removed or its severity lessened. Let us consider these two categories of teratomata in some detail.

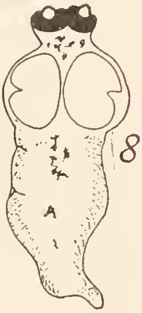
(A) Forms Resulting from Differential Inhibition.

In fish monsters, whether they are the result of chemical, physical, or biological (hybridization) inhibitors, the most prevalent abnormalities are associated with the eyes and with the heart. The teratomata resulting from all three types of inhibiting agents are strikingly similar, so I shall from now on confine my description and discussion to monsters produced by heterogenic hybridization. In any heterogenic cross the least abnormal forms are always those that have something slightly abnormal or sub-normal about the eyes or the heart. We must conclude then that the primordia of these two structures are peculiarly susceptible to agents that retard metabolic rate.

Ophthalmic Terata.—Eyes in the least extreme cases may be either a little too small, a little too close together, of unequal size, or asymmetrical in position. In the most extreme cases the eyes may be wanting, or very poorly differentiated; the eyes may be fused more or less completely (various grades of cyclopia) or there may be a single unilateral eye. Every intergrade between the least extreme and the most extreme ophthalmic terata may be readily found in almost any hybrid experiment. Why in a single batch of eggs there should be so highly diversified a result is not known, but it must be due to physiological (age, maturity, etc.) differences in the individual eggs or sperm, or a combination of both.

In general we may conclude that suppressed eyes, either bilateral or unilateral, are the result of inhibition primarily of the apical end of the apico-basal gradient, while eyes too close together or fused in the median line (cyclopic terata) are the result primarily of inhibition of the median portion of the mesio-lateral axis.

Cardiac Terata.—All grades of heart abnormalities occur in heterogenic crosses. The less extreme cases are those in which the heart development is belated relatively to the other bodily regions, or in which there may be a failure of the whole cardiac mechanism to become inclosed within the body cavity. In such cases the heart remains outside the body and there is usually a much enlarged pericardium. In the most extreme cases the heart never develops, or becomes a mere pulsating strip of muscular tissue running from the body to the yolk of the egg across the pericardium. These "string-hearts" are among the commonest terata to be found in heterogenic hybrids. We have no choice but to conclude that, although the heart is not very close to the apical end, and is ventral rather than dorsal in position, that it is an organ of relatively high rate of metabolism and shows extreme susceptibility to retarding agents. It is undoubtedly true that the heart and blood vessels form an axiate system of their own, which is somewhat independent of general somatic axes. The attached or arterial end (ventricles) of the heart is the apical point of this axis and is the part most susceptible to retarding agents. When this end of the heart is inhibited the whole circulatory system will be rendered functionless, though the basal parts may be fairly well developed. When the apical parts are only slightly abnormal the basal parts may be fully normal, but when the apical parts show extreme abnormality the basal parts are usually abnormal but less so than the apical.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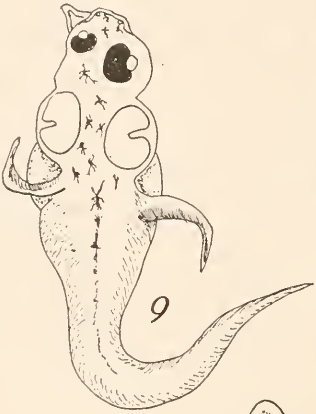
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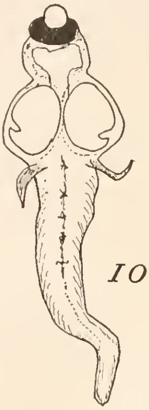
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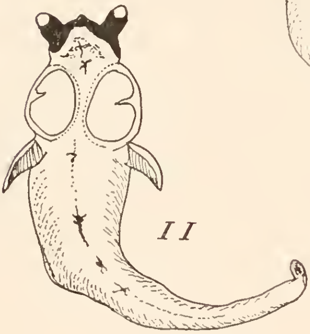
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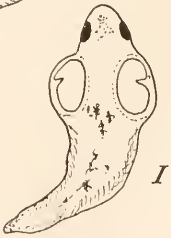
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(B) Forms Resulting from Differential Acclimation or Recovery.

Of somewhat less frequent occurrence than the above described types of fish hybrid teratomata are those in which the apical and mesial parts have become acclimated to (or adjusted to) the materials brought in by the foreign sperm. The less extreme types are those in which the head is relatively large and wide as compared with the trunk and tail. Next comes a whole series of types in which first the tail and then the trunk are inhibited more or less completely, and even heads without trunks but with beating hearts are common. The most extreme cases are those in which isolated eyes or isolated hearts grow upon an otherwise undifferentiated blastoderm (Fig. 14). I have found several examples of isolated eyes that could be distinguished only by the presence of a lens and of pigmented retinal cells. Also isolated hearts may be reduced to small pulsating "drums" of tissue in which there is no axiate elongation. According to the axial gradient theory not only are the apical points most susceptible to inhibitors, but under certain circumstances, such as long exposure to low concentrations, they have the highest capacity for recovery. That is why eyes and hearts, the apical parts of the two axiate systems, show the highest capacity for recovery and are sometimes the only parts that do recover after long and general inhibition. A very common type of teratomata is one in which the whole body is decidedly abnormal, inhibited no doubt at an early stage, but in which recovery of the apical structures has occurred to various degrees, so that we have forms in which the only highly differentiated parts are apical parts. Such embryos are shown in Figs. 13 and 14.

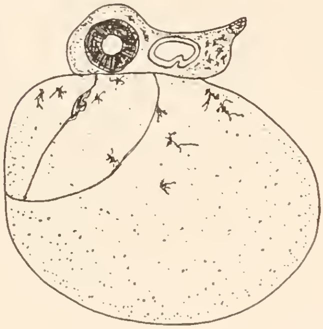
Child ('16) distinguishes between forms resulting from differential acclimation, forms resulting from differential recovery, and forms resulting from differential inhibition with general recovery. While I believe that the forms resulting from hybridization are identical to those produced by chemical inhibition, I am not able to say in any particular case whether a form is one or the other of these three types. My judgment is that the isolated eyes and hearts are forms resulting from differential recovery and that the forms with comparatively well developed head and small or rudimentary body are the result of differential acclimation. After all, the results of acclimation and recovery even in controlled chemical experiments are so nearly identical that, were it not for the differences in experimental method used, they would be indistinguishable.

On the whole then we can distinguish clearly only the two types of monsters:

(*a*) Forms with inhibited apical parts and relatively well developed basal parts—the results of differential inhibition.

(*b*) Forms with inhibited basal parts and relatively well developed apical parts—the results of differential acclimation or recovery.

None of the forms of fish hybrids with which I am acquainted fail to fall into one of these two categories and to receive a logical explanation on the basis of the axial gradient theory. It is my belief that this theory offers the best explanation of teratological development in fish and in all other axiate forms, including man.



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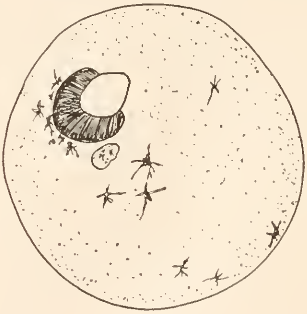

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As a concrete illustration of the application of the theory to a single experiment, I shall offer the details of an intensively studied hybridization experiment, the one referred to earlier in the paper in which the three lots of hybrid eggs were separated on the basis of the rate of early cleavage into three lots A, B and C. This experiment dealt with hybrids of *Fundulus heteroclitus* ♀ × *Scomber scombrus* (mackerel) ♂.

Figs. 1–4 show types of teratomata in lot A. These embryos are frequently almost normal up to an advanced stage and a very few hatch as perfectly normal larvæ. A considerable percentage of embryos develop a circulation but have comparatively small eyes as in Fig. 1. Others have a proboscis and the eyes both look forward as in Fig. 2. Still others have a very narrow head with small eyes and the heart is a string-heart as in Fig. 3. Others, finally, are cyclopic forms in which the mesio-lateral development of the head has been more seriously inhibited than the apico-basal (as in Fig. 4).

The largest and most varied assemblage of monsters is found in Class B in which the rate of cleavage was intermediate between the lowest and highest rate. Types of embryos of this group are shown in Figs. 5–12, which are camera lucida drawings made when embryos were two weeks old. In all of these the whole body from head to tail is abnormal (compare with the embryos of A in which the tail is nearly normal). Ophthalmic terata are most common here. Fig. 5 is microphthalmic and a little asymmetrical and the body and tail are fairly normal; pectoral fins are also developed and the otic vesicles are only slightly enlarged. Fig. 9 has decidedly asymmetrical eyes, one very small; the jaws, pectoral fins, etc., are also asymmetrical, but the tail is fairly well developed. Fig. 11 shows a peculiar type of stalked-eye, which is rather common; body is only slightly abnormal; pectoral fins normal. Figs. 6 and 10 are two types of cyclopic monster with reduced and shortened tail, but with enlarged otic vesicles and good pectoral fins. Fig. 8 is a synophthalmic type with enlarged otic vesicles and much reduced body. Fig. 12 is a common form with much reduced eyes, well developed otic vesicles and small body. Fig. 7 is an eyeless type in which the otic vesicles seem to have usurped the place of the eyes in the body; the body is fairly well developed. All of the types here shown had the heart beating. All of these forms I look upon as products of acclimation after an early partial inhibition. They were not as much retarded as those in lot C. I look upon the enlargement of the otic vesicle as the result of the suppression or partial suppression of the dominant region anterior to it. The otic region now is to some extent physiologically isolated and thus released to grow to a larger size than normal.

I shall show only two types out of a very large number found in lot C in which the slowing of the cleavage rate was most pronounced. Fig. 13 shows a common type which is practically a head without a body. The little up-turned stump is all there is of the body; the heart is still beating; the eyes are in an advanced stage of differentiation. Fig. 14 shows a case of solitary or isolated eye. No other tissues are differentiated. I look upon these as cases of recovery of the apical regions after rather severe early inhibition. There is no hard and fast line to be drawn between the three classes of monsters shown.

It may be said in closing that each type of hybrid cross differs from others in the particular array of monsters displayed. In some there is never cyclopia, but always microphthalmia. In some the extreme recovery types, like that shown in Fig. 13, are much the most common. In many respects the *F. heteroclitus* ♀ × mackerel ♂ cross is the best adapted for the study of the morphogeny of teratomata because of the very wide range of forms produced.

In conclusion I wish to express my appreciation of the far-reaching applications to morphogenetic phenomena of the axial gradient idea as worked out by Child. Few generalizations known in biological science serve to rationalize so wide a range of developmental phenomena.

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RESULTS OF CROSSING EUSCHISTUS VARIOLARIUS AND EUSCHISTUS ICTERICUS WITH REFERENCE TO THE INHERITANCE OF TWO EXCLUSIVELY MALE CHARACTERS.

KATHARINE FOOT AND E. C. STROBELL.

The two exclusively male characters selected for study in this cross are first, the black or dark brown spot on the male genital segment of *Euschistus variolarius* and, second, the intromittent organ, which differs markedly in length in the two species.

In two recent papers[1] we described in detail the transmission of the above-mentioned exclusively male characters in a cross between *Euschistus variolarius* and *E. servus* and the results from the study of the transmission of these two characters in the cross of *E. variolarius* with *E. ictericus* are merely a confirmation of the results obtained from the cross of *E. variolarius* with *E. servus*. The results from the latter cross were published first because they were more satisfactory, for the reason that the spot which is so pronounced on the male genital segment of *variolarius* is entirely absent from the male segment of *E. servus*, whereas it is not entirely absent from the genital segment of *E. ictericus*. Although *ictericus* is described as having no spot on the genital segment, a faint spot is frequently present. This is not easily determined until the segment is pulled out and closely examined, but the spot, if present, can then be clearly seen, though it is not nearly so pronounced as that of *variolarius*. (Compare photos 61 and 62.) Although this spot is not a constant feature in *ictericus*, it is present often enough to make it impossible to determine how much of the inheritance of the spot in the hybrids is due to the female *variolarius* and therefore the results from this cross are less decisive, on this point, than the results from the cross with *servus*.

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[1] "Results of Crossing *Euschistus variolarius* and *Euschistus servus* with Reference to the Inheritance of an Exclusively Male Character," *Journ. Linn. Soc. London*, Zoöl., Vol. XXXII, 1914.

"Results of Crossing Two Hemipterous Species, with Reference to the Inheritance of Two Exclusively Male Characters," *Journ. Linn. Soc. London*, Zoöl., Vol. XXXII, 1915.

The intromittent organ, however, is a chitinous spiral which can be dissected out and accurately measured, making it possible to determine the exact size relations in the hybrid. We shall, therefore, first describe the results obtained from a study of the inheritance of this organ and shall then more briefly state the less definite results obtained from a study of the genital spot.

Dr. Eltringham's discovery of the difference in the length of the intromittent organ between the two species *E. variolarius* and *E. servus* (Foot and Strobell, '15) led us to expect a difference in the length of this organ between the two species *E. variolarius* and *E. ictericus*. The three species do, in fact, differ greatly in this character. At a magnification of twenty diameters, the mean length of the intromittent organ of *E. servus* is 166.41 mm. of *E. variolarius*, 96.70 mm. and of *E. ictericus*, 60.64 mm.

Our cross-breeding experiments with *variolarius* by *ictericus* differ in important details from our experiments with *E. variolarius* by *E. servus*. In the latter we succeeded in raising to maturity, eleven males and 16 females of the F_1 generation, and from these we were able to raise a large number of the F_2 generation (204 males and 249 females). In the *variolarius* × *ictericus* cross, on the contrary, we succeeded in raising only one specimen of the F_1 generation (a female). We were successful, however, in crossing this F_1 female with a pure *ictericus* male and we raised from this back-cross 76 males and 73 females. These results are a gratifying supplement to our cross-breeding experiments with *variolarius* and *servus*, for in the latter, a back-cross was commenced so late in the season that we were able to secure only 18 males and 8 females.

Details of Crossing Euschistus variolarius × Euschistus ictericus.—The three female *variolarius* used for this cross were hatched in our laboratory, and immediately after the fifth moult were caged with three male *ictericus* which were also hatched and reared in the laboratory. The parents of the three females were two of ten specimens of *variolarius* collected at White

Plains, N. Y., April 16, 1911, by Mr. de la Torre Bueno. These bugs were placed in a cage in our laboratory and, four days later, one pair mated, and while mating were transferred to a separate cage, in which they were kept during the entire breeding season, until the female died.[1]

Three of the female offspring of this pair were used for the cross-breeding experiments with *ictericus*. They reached the winged stage on the following dates: July 3, one female reared from the group of 4 eggs deposited May 22; July 15, two females reared from the group of 29 eggs deposited June 9. The three male *ictericus* that were caged with these three *variolarius* females were reared from eggs deposited in our laboratory May 22 and June 2. These eggs were deposited in a cage containing ten *E. ictericus* specimens that had been collected a few days before by Dr. Knab in a swamp near Washington, D. C. One of the males from these eggs reached the winged stage July 7 and two, July 15 and the three were caged with the three above-mentioned *variolarius* females.

August 12 a pair of these bugs mated and while mating were transferred to a separate cage (cage 5). The record of this pair shows that they mated 6¼ hours[2] and did not mate again while they were under observation (from August 12 to November 24, 1911) when the female died. On August 15, 7 eggs were deposited, none of which developed. On August 18, 4 eggs were deposited one of which hatched and was raised to maturity—this being the F_1 female which we mated the following spring to a pure *ictericus* male. On August 25, 23 eggs were deposited, none of which developed. (See photo 63 for the male of this pair.)

A second pair mated August 19 and this pair also was isolated (cage 13). A group of 8 eggs was deposited by this second pair August 22 and a group of 18 eggs on August 25 but none of these 26 eggs developed.

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[1] The record of the breeding period of this pair of *variolarius* was published in our report of the results of crossing *E. variolarius* × *E. servus*. This record is entered as "cage 2, 1911." Foot and Strobell, '14, *Journ. Linn. Soc. London, Zoöl.*, Vol. XXXII, p. 362.

[2] The length of time for each mating can be given only approximately as observations were made not oftener that three or four times each hour, during the day and three or four times during the night.

The last pair of bugs of the original three pairs never mated, although they were kept under observation until October 11. They were then added to a cage containing eight specimens of the same cross (4♀ and 4♂) which we had caged to carry through the winter.

The record of the first pair shows that from August 15 to August 25, 34 eggs were deposited and that only one of these developed. If we add to these the above-mentioned 26 eggs from the second pair of this cross, we find that out of 60 *variolarius* eggs, that were presumably fertilized by *ictericus*, only one developed and hatched.

Comparing these results with those of the cross between *variolarius* × *servus*, we find that in the latter a very much larger proportion of eggs developed, for out of a total of 120 eggs, 37 developed and 32 of these hatched.

This contrast between the results of the two crosses is greatly increased if we add the number of eggs deposited by the *variolarius-ictericus* cross of the summer of 1912. As stated above we caged through the winter of 1911–12 five female *variolarius* and five male *ictericus*. All of these were reared in the laboratory from the same stock as those of the 1911 pairs. Only one of these mated the next season (cage 12, 1912). Of the 53 eggs which were deposited between June 11 and July 15 not one developed. We believe we might have had better success with wild males; but we failed in our efforts to get them in the spring of 1912. In summing the results we find that 113 eggs in all were deposited by the three *variolarius* females that were crossed with *ictericus* and that only one of these 113 eggs hatched; the remaining 112 did not show even the initial stages of development. This one egg that developed was deposited August 18 and was not hatched until August 28, as the weather was unseasonably cold. The nymph (a female) reached the winged stage September 26. We succeeded in keeping this hybrid in good condition through the winter of 1911–12, and the following June she mated with a pure *ictericus*. The record of this pair is as follows:

RECORD OF CAGE 3, 1912.[1]

F_1 Hybrid ♀ (from *E. variolarius* × *E. ictericus*) and pure *E. ictericus* ♂—one pair.

June

15 3.45 P. M. mating. Continued to mate 17 hours.

21 20 eggs (in two groups) 15 survived first moult.

24 8 eggs. 7 hatched.

28 6 eggs. 5 hatched.

29 13 eggs. 12 hatched.

33 reared to winged stage (16♂ and 17♀).

When some of the nymphs from the above four sets of eggs reached the third or fourth moult, they were transferred to a large cage and therefore some specimens from each of these four sets were combined and thus the individuality of each set was lost.

July

5 12 eggs. 10 hatched. 9 reared to winged stage (3♂ and 6♀).

6 14 eggs. All hatched. 13 reared to winged stage (5♂ and 8♀).

8 7 eggs (on wire top of cage ⎫ 12 hatched. 9 reared to winged stage (2♂
9 6 eggs (on wire top of cage ⎭ and 7♀).

12 12 eggs. (5 of them on wire top of cage) 10 hatched. 9 reared to winged stage (5♂ and 4♀).

16 8 eggs. All hatched. 7 reared to winged stage. 5♂ and 2♀.

21 5 A. M. 6 eggs (on wire top of cage). All hatched. All reared to winged stage. (4♂ and 2♀.)

21 5 A. M. mating. Continued to mate 21 hours.

24 18 eggs. All hatched. 16 reared to winged stage. (8♂ and 8♀.)

30 22 eggs (in 5 groups). 21 hatched. 18 reared to winged stage. (10♂ and 8♀.)

August

6 5 eggs ⎫
9 6 eggs ⎭ All hatched. 9 reared to winged stage. (5♂ and 4♀).

13 14 eggs (in 4 groups). 12 hatched. 8 reared to winged stage. (5♂ and 3♀.)

17 12 eggs (in 2 groups). 11 hatched. Later added to nymphs from eggs deposited August 28.

22 3.30 P. M. mating. Continued to mate 48 hours.

28 6 eggs (scattered on wire top of cage). 5 hatched. Later added these to nymphs from eggs deposited August 17. 12 reared to winged stage. (8♂ and 4♀.)

30 Killed both the male and female. Both were inert and apparently nearly dying. (The female has had only four legs since July 12.) Preserved in glycerine (tube 33). See photo 64 for the male.

All the females of this pair and 20 males are preserved as pinned specimens; the rest of the males are preserved in glycerine. See photos 65–69 for twenty-five specimens.

[1] The data of this record are taken from our notes from which we have selected only the essential items, omitting such details as the dates of hatching, the dates when the five moults occured and the number of nymphs that survived each moult. The eggs hatch from five to seven days after deposition, this variation depending largely on the temperature .

A comparison of the above record with those of pure *variolarius* shows that in this back-cross the relation between mating and deposition of eggs is not normal, for, as a rule, eggs are deposited not oftener than once or twice between two matings, whereas in this cross, eggs were deposited eleven times between the first two matings and six times between the last two matings. The number of eggs, however, that were deposited between June 21 and August 28 is above our average for pure *variolarius* and a normal proportion of these eggs developed. 195 eggs were deposited and 177 of these hatched. 149 were reared to the winged stage. (76♂ and 73♀.) All of these females are preserved as pinned specimens and the males are preserved either as pinned specimens or in glycerine.

Intromittent Organ.—This organ is a chitinous spiral easily dissected from the genital segment of the males (photos 1 to 60).

In accordance with Dr. Eltringham's suggestion, the entire male segment was first softened in warm caustic potash, until the intromittent organ was sufficiently pliable to be dissected out without breaking.

Only a single organ was mounted on a slide, and the slide was so indexed that the insect from which the organ was taken could be identified and therefore, the relation of the intromittent organ to any other character could be satisfactorily studied in one and the same insect. This was possible in the case of the genital spot, for the genital segment of all the specimens which were preserved in glycerine had been photographed before the segment was removed for dissection.

Each intromittent organ being mounted in balsam, it was photographed at a magnification of 20 diameters, and all measurements were made from these photographs. The actual length, therefore, of each organ is one-twentieth of the length recorded in this paper.

Measurements from such photographs of the intromittent organs of *E. variolarius*, *E. ictericus* and of the offspring from the back-cross are given in the following three tables.

Table I. gives the lengths of the intromittent organs of 62 specimens of *E. variolarius.* This table is quoted from our report of results from the cross between *E. variolarius* and *E. servus.* (Foot and Strobell, '15, see footnote, p. 322.)

Table II. gives the lengths of the intromittent organs from 32 specimens of *E. ictericus.*

Table III. gives the lengths of the intromittent organs of 71 offspring from the back-cross of a pure *ictericus* male with the one F₁ female we succeeded in raising from crossing *E. variolarius* ♀ by *E. ictericus* ♂.

As the intromittent organ of *E. ictericus* is much shorter than that of either *E. variolarius* or *E. servus*, it was much less difficult to get an accurate measurement of the length of the organ in *icterícus* and this was true also of the offspring from the

TABLE I.

Lengths of intromittent organs from 62 specimens of *Euschistus variolarius* × 20 diams.

85.5 mm.	1
87 mm.	1
88 mm.	1
89 mm.	1
90 mm.	1
91 mm.	1
92 mm.	2
93 mm.	4
93.5 mm.	1
94 mm.	6
94.5 mm.	2
95 mm.	1
95.5 mm.	2
96 mm.	7
96.5 mm.	1
97 mm.	1
97.5 mm.	1
98 mm.	6
98.5 mm.	1
99 mm.	2
100 mm.	7
100.5 mm.	1
101 mm.	4
102 mm.	3
104 mm.	1
104.5 mm.	2
106 mm.	1
5,996 mm.	62

Mean length = 96.70 mm.

TABLE II.

Lengths of intromittent organs from 32 specimens of *Euschistus ictericus* × 20 diams.

54.5 mm.	1
55 mm.	1
58 mm.	3
59 mm.	1
60 mm.	8
60.5 mm.	3
61 mm.	2
62 mm.	6
62.5 mm.	2
63 mm.	3
64 mm.	1
64.5 mm.	1
1,940.5 mm.	32

Mean length = 60.64 mm.

back-cross. We used, however, the same method as for the longer and more complicated coils of *servus*, *i. e.*, photographing each intromittent organ at a magnification of 20 diameters, and measuring the photographs with a small pair of architect's dividers. (For details see Plates I.–III., p. 337.)

Table III.

Lengths of intromittent organs from 71 specimens of offspring from back-cross *i. e.*, F_1 ♀ (from *E. variolarius* ♀ × *E. ictericus* ♂) by *E. ictericus* ♂ × 20 diams.

60 mm.	2
62 mm.	2
62.5 mm.	1
63.5 mm.	1
64 mm.	4
66 mm.	8
66.5 mm.	3
67 mm.	1
68 mm.	7
68.5 mm.	1
69 mm.	7
70 mm.	10
70.5 mm.	2
71 mm.	3
72 mm.	7
72.5 mm.	2
73 mm.	1
73.5 mm.	1
74 mm.	3
74.5 mm.	1
75 mm.	1
76 mm.	2
76.5 mm.	1
4,897.5 mm.	71

Mean length = 68.97 mm.

Genital Spot.—A study of the inheritance of the genital spot in the offspring from the back-cross of *variolarius-ictericus* is of interest merely as furnishing additional data in support of the results given by the crosses of *variolarius* by *servus*. These results, however, are by no means as definite as those from the intromittent organ, for the dimensions of the genital spot can be only relatively stated, they cannot be accurately measured as in the case of the length of the intromittent organ. The influence of each parent in the transmission of the genital spot in the hybrids can, therefore, be ascertained only approximately and the case is further complicated in the *ictericus* cross by the fact that in *E. ictericus*, unlike *E. servus*, a genital spot is not wholly absent.

Photo 61 is of seven male specimens of *E. variolarius* showing on each genital segment, the brown or black spot characteristic of the *variolarius* male, and photo 62 is of seven specimens of *E. ictericus* showing the faint indication of a genital spot which is characteristic of nearly all *E. ictericus* males. As stated above, the spot, when present, is so indistinct that in most cases it is easily overlooked unless the segment is pulled out and closely examined, and this is perhaps why this species is described as having no spot on the genital segment. Van Duzee's description is as follows:

"*Euschistus icterictus* Linn.

"Found in the Northern States and Canada across the whole width of the continent. It is generally to be found on sedges in swampy spots or along the borders of streams or other bodies of water. It may be distinguished from the foregoing [*E. variolarius*] by the calloused ruga connecting the humeri which are more produced than in *variolarius*, and the genital segment of the male wants the black spot found in that species."[1]

Although the presence of a faint spot in *ictericus* is an embarrassing factor in comparing the results of the *variolarius-ictericus* cross with those of the *variolarius-servus* cross, it by no means obscures the fact that the evidence from the two crosses is in complete harmony.

The *servus* crosses demonstrated that the genital spot was transmitted through the female of the first cross, and this is supported by the *ictericus* back-cross. The inheritance from the *variolarius* female of the first cross can be appreciated by comparing the faint spot of the pure *ictericus* specimens of photo 62 with the spot in some of the back-cross offspring, for example the first specimen of photo 65, the second specimen of photo 66, the third and last specimens of photo 69. In these the spot is almost as strong as that of the pure *variolarius* males and much stronger than any of the genital spots of the pure *ictericus* males.

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[1] Our *Euschistus icterictus* specimens were identified by J. R. de la Torre Bueno.

The *variolarius-servus* crosses further demonstrated that the genital spot was transmitted directly from the male and this is also supported by the *variolarius-ictericus* back-cross. The *ictericus* inheritance is shown in all the photographed specimens in which the spot is quite as insignificant as the faintest spot in the pure *ictericus* specimens.

Although the harmony in results with those of the *servus* experiments is evident, the factor of an indefinite spot in *ictericus* makes a detailed comparison less satisfactory and for this reason we shall discuss the results from the two back-crosses in relation to the intromittent organ rather than the genital spot. These two exclusively male characters (as we demonstrated by the *servus* crosses) give exactly the same evidence, in every detail, in their bearing on current theories under discussion.

Results and Discussion.

The results obtained from this back-cross, F_1 ♀ (from *variolarius* × *ictericus*) by *ictericus*, are so nearly a repetition of those obtained from the back-cross of F_1 ♀ (from *variolarius* × *servus*) by *variolarius*, that they would seem scarcely to merit separate publication: but for the fact that the results obtained from the few specimens secured from the latter cross were of small scientific value, until supported by more data, and such data (from *ictericus*) were not in shape for publication when the results of the *servus* cross went to press.

One point of interest in the back-cross of F_1 ♀ by *variolarius* was that the offspring did not show a Mendelian type of inheritance, but that the length of the intromittent organ appeared to be a quantitative response to the proportion of inheritance from the two species.

In attempting to measure the strength of the inheritance in this cross, we found that the effect on the length of the organ corresponded almost exactly with the relative amount of *variolarius* to *servus* in the offspring. This was 3 *variolarius* to 1 *servus*, as *variolarius* was first crossed with *servus* and a male *variolarius* used for the back-cross with the F_1 female. In our report of this cross we stated the results as follows:

"If we measure the amount of the *variolarius* inheritance in this back-cross, we find an astonishing agreement between the theoretical expectation and the actual result. The relative amount of *variolarius* to *servus* in the offspring of this back-cross is 3 to 1, and we should expect, therefore, the mean length of the intromittent organ of *servus* to be reduced by 75 per cent. of the difference between the mean lengths in *variolarius* and *servus*. This difference is 69.71 mm., 75 per cent. of this being 52.28 mm. Deducting this from the mean length of the organ of *servus* (166.41 mm.) would leave 114.13 mm. as the mean length of the organ of the offspring from this back-cross. The mean length is in fact 113.47 mm., this being only 0.66 mm. less than the calculated expectation."[1]

If our results had followed a Mendelian type of inheritance, assuming the *servus* length of organ to be dominant, we should expect the mean length of the intromittent organ to be approximately 131.33 mm. instead of being the above simple expression of the proportion of inheritance from the two species.

This result would seem scarcely to merit serious consideration, as it was based on the inheritance from only 18 males; but from the back-cross reported in the present paper (*variolarius-icterícus*) we succeeded in getting 76 males and these repeat the evidence given by the 18 males of the first experiment.[2]

The two experiments are entirely similar except that in the first experiment the back-cross was made with a male of the same species as the female of the first cross (*variolarius*) and in the second experiment the back-cross was made with a male of the same species as the male of the first cross (*icterícus*).

If we look for an agreement in results from the two experiments, we should expect the mean length of the intromittent organ of the 71 males from the second back-cross to show a like quantitative response in inheritance to that in the case of the 18 males of the first experiment.

In this second back-cross we have 3 parts *icterícus* to 1 *variolarius*, and we should expect the mean length of the intromittent organ of *variolarius* to be reduced by 75 per cent. of the difference between the mean lengths in *ictericus* and *variolarius.* This difference is 36.06 mm., 75 per cent. of this being 27.04 mm. Deducting this from the mean length of the organ of *variolarius* (96.70 mm.) would leave 69.66 mm. as the mean length of the organ from this back-cross. The mean length is in fact 68.97 mm., this being 0.69 mm. less than the calculated expectation. In both back-crosses the slightly stronger inheritance is in favor of the shorter type of intromittent organ and from the species giving 75 per cent. of the inheritance.

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[1] Foot and Strobell, '15, "Results of Crossing Two Hemipterous Species with Reference to the Inheritance of two Exclusively Male Characters," *Journ. Linn. Soc. London*, Zoöl., Vol. XXXII., p. 474, 1915.

[2] The intromittent organs of 71 of these 76 males were measured. See page 329.

The 71 males from this back-cross further support the results of the first experiment as follows:

The back-cross (*variolarius-servus* by *variolarius*) showed that the length of the intromittent organ was transmitted directly from the male and also indirectly through the female, and that therefore the so-called male- and female-producing spermatozoa do not differ functionally in their transmission of a character so exclusively male as the intromittent organ.

Its direct transmission by the male was proved by the decrease in the mean length of the organ of the offspring from the back-cross as compared with that of the F_2 hybrids and it is further shown by the F_1 generation of the *variolarius-servus* crosses. The facts are as follows:

Mean length of the organ—

In the *variolarius* species	96.70 mm.
In the *servus* species	166.41 mm.
In the F_1 hybrids	124.9 mm.
In the F_2 hybrids	124.42 mm.
In the offspring from the back-cross	113.47 mm.

The *increase* in the mean length of the organ in the F_1 hybrids as compared with that of the *variolarius* species proves the direct transmission from the male parent, and the *decrease* in the mean length of the organ in the offspring from the back-cross as compared with that of the F_1 and F_2 generations shows again the direct influence of the male parent, the *servus* male of the first cross increasing the length towards that of the *servus* species and the *variolarius* male of the back-cross reducing it towards the *variolarius* species.

These results are supported by the *variolarius-ictericus* back-cross though the evidence of the *direct* influence of the male parent might be questioned in this cross, as we did not succeed in securing any F_1 or F_2 males, and this would leave open the question whether the *ictericus* inheritance in these offspring was due in part to direct inheritance from the male of the back-cross or wholly due to indirect inheritance through the F_1 female. In the *servus* experiments, on the contrary, the direct influence of the male in both the first cross and the back-cross is beyond question.

Evidence of the transmission of this exclusively male character through the female is given in both back-crosses, but in the *variolarius* by *servus* experiments it is most conclusively shown by the mean length of the intromittent organ of the F_1 generation, where the inheritance from the pure *variolarius* female of the first cross is clearly in evidence.

In the *ictericus* experiment, the inheritance from the pure *variolarius* female of the first cross is shown in the increased mean length of the intromittent organ of the offspring of the back-cross as compared to that of the pure *ictericus* species. The facts are as follows:

Mean length of the organ—

In the *variolarius* species	96.70 mm.
In the *ictericus* species	60.64 mm.
In the offspring from the back-cross	68.97 mm.

A further point for consideration in the two back-crosses is the fact that in the first (*variolarius-servus*) the 25 per cent. inheritance is from the *male* of the first cross (*servus*) and in the second (*variolarius-ictericus*) the 25 per cent. inheritance is from the *female* of the first cross (*variolarius*). As in the one case, this one quarter inheritance was received directly from the male (*servus*) and in the other case indirectly through the female (*variolarius*), and as these have so nearly the same value in the offspring from both back-crosses, we would seem to have the strongest evidence that the so-called male- and female-producing spermatozoa do not differ quantitatively in their transmission even of a character so exclusively male as the intromittent organ.

In the *variolarius-servus* by *variolarius* back-cross we have direct evidence that this exclusively male character—the length of the intromittent organ—is transmitted by the so-called female-producing spermatozoa, for the *servus* inheritance is from the F_1 female, which must have developed from an egg fertilized by a female-producing spermatozoön of *servus*.[1]

Thus the evidence that not only the so-called male-producing spermatozoa but also the so-called female-producing spermatozoa can transmit even such an exclusively male character as the intromittent organ, and the fact that these so-called sex-determining spermatozoa do not differ functionally in the transmission of such a character certainly justifies much scepticism as to that sex-determination hypothesis which is based on the assumption of male- and female-producing spermatozoa.

In the reports of our *variolarius* by *servus* experiments we have repeatedly discussed the bearing of the results on the interesting and ingenious hypotheses which attempt to locate Mendelian factors not only in the chromosomes as such, but in definite chromosomes and in definite areas of definite chromosomes, and we have pointed out that the evidence from our results was in direct contradiction to such hypotheses and, further, that the evidence, as stated above, was against the hypothesis that assigned the rôle of sex-determination to the "sex-chromosomes" of the so-called male- and female-producing spermatozoa. As the evidence bearing on these questions is simply repeated in this *variolarius* by *ictericus* cross, we would (to avoid repetition) refer our readers to a recent paper[2] where we have briefly discussed the evidence in relation to these questions.

We feel almost like apologizing for our persistent criticism of these ingenious chromosome hypotheses so brilliantly launched by Morgan and his pupils[3] and so vigorously advocated by Wilson.[4]

Our scepticism was perhaps primarily due to fourteen years' patient study of the chromosomes of *Allolobophora fœtida*, these years of investigation of one form forcing us to the conviction that the chromosomes share a variability that is admittedly a characteristic of other organs of the cell and a characteristic of all organic structures. Such convictions as to the morphological nature of the chromosomes made it impossible to have faith in any hypotheses that were based on the type of individuality of the chromosomes demanded by the theories in question.

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[1] For the sake of the argument we must assume, for the moment, the existence of male- and female-producing spermatozoa.

[2] Foot and Strobell, *Journ. Linn. Soc.*, London, 1915, pp. 475–486.

[3] A complete list of the literature by Morgan and his pupils is given in their recent volume, "Mechanism of Mendelian Heredity," Morgan, Sturtevant, Muller and Bridges. Henry Holt & Company, 1915.

[4] A forceful presentation and endorsement of these hypotheses was recently given by Wilson in his Croonian Lecture. *Proceedings Royal Soc.*, ser. B, Vol. LXXXVIII., No. B 603.

In a recent paper, Trow[1] gives an able and searching criticism of one of these hypotheses and he has the sympathy of all sceptics when he voices the pressing need of an accurate knowledge of the chromosomes, especially of the maturation divisions where alone can be found the cytological support essential to a foundation for such a superstructure of imaginary architecture. This need is further voiced by Bateson[2] in his report on a recent volume by Morgan and his pupils.[3] Of the maturation prophases he says: "That twisting takes place in many types, especially Amphibia, is clear; but neither the figures reproduced from Janssens nor the originals from which they are taken—still less the very fragmentary observations of both Stevens and Metz from *Drosophila*—provide more than a slender support for this most critical step in the argument. It is to be hoped that the authors will before long tell us exactly upon what evidence they are here relying."

It is well known by cytologists that, with our present methods of technique, an accurate knowledge of the maturation divisions of *Drosophila* is impossible and one is led to suspect that this ignorance as to the chromosomes leaves the imagination a freedom that perhaps would not be possible if curbed by more hard facts.

[1] "A Criticism of the Hypothesis of Linkage and Crossing Over," *Journ. Genetics*, Cambridge, England, Vol. 5, No. 4, 1916.

[2] *Science*, N. S., Vol. XLIV., No. 1137, p. 536.

[3] "The Mechanism of Mendelian Heredity."

EXPLANATION OF PLATES I.–III.

We photographed each intromittent organ at a magnification of 20 diameters and prints on matte paper were made from these negatives. Then each subject was accurately measured with a small pair of architects' dividers fitted with no. 9 needle points, and set at 2 mm. The measurements were made on the matte prints so that each division of 20 mm. could be indicated by a pencil mark and numbered. The measurements were taken from the distal end of the intromittent organ to the point where the thick part of the coil enters the gland. The coil is most easily dissected off at this point (*e. g.*, photos 3, 5, 6, 8, etc.) but in those cases where part of the canal within the gland has been preserved (*e. g.*, photos 1 and 2) the point from which the measurement was made may be readily determined, for the part within the gland is transparent and quickly tapers to a finer canal which apparently extends through the entire gland. The transparency of that portion of the canal within the gland is much clearer in the preparations than in the photographs, for when the chitin has a yellow tinge it appears black and opaque in the photograph.

In many specimens we have left a small piece of the gland at the point where the intromittent organ enters (*e. g.*, photos 7, 9, 10, 12, 14, 19, etc.).

Plate I.

Photo 1. Intromittent organ of *Euschistus variolarius*—the father of the *E. variolarius* female that was fertilized by the pure *E. ictericus* male.

Length of intromittent organ 96 mm.

Photos 2, 3 and 4. Intromittent organs of three brothers of the *E. variolarius* female that was fertilized by the pure *E. ictericus* male. From this cross we raised the F1 female which was back-crossed with a pure *E. ictericus* male and produced the offspring described in this paper.

Length of the intromittent organ of photo 2, 100 mm.—of photo 3, 94 mm.—of photo 4, 99 mm.

Photos 5 to 8. Typical specimens of the intromittent organ of *Euschistus variolarius*.

Length of the intromittent organ of photo 5, 93 mm.—of photo 6, 93.5 mm.—of photo 7, 97.5 mm.—of photo 8, 98 mm.

Photo 9. Intromittent organ of the *Euschistus ictericus* of the original cross (cage 5, p. 324). This male *ictericus* was raised in our laboratory and fertilized the *Euschistus variolarius* female from which we obtained the F1 female used for our back-cross experiment.

Length of the intromittent organ 60 mm.

Photo 10. Intromittent organ of the *Euschistus ictericus* that fertilized the F1 female . . . (the father of all the offspring from our back-cross experiment). (See record, p. 326.)

Length of the intromittent organ 63 mm.

Photos 11, 12 and 13. Intromittent organs of three of the original stock of *Euschistus ictericus* that we received from Washington, D. C., May 18, 1911.

Length of the intromittent organ of photo 11, 62 mm.—of photo 12, 60 mm.—of photo 13, 62.5 mm.

Photo 14. Intromittent organ of an *E. ictericus* male that was raised in our laboratory in 1911, kept through the winter, and in 1912 mated with a pure *E. variolarius* female. (See account of cage 12, p. 325.)

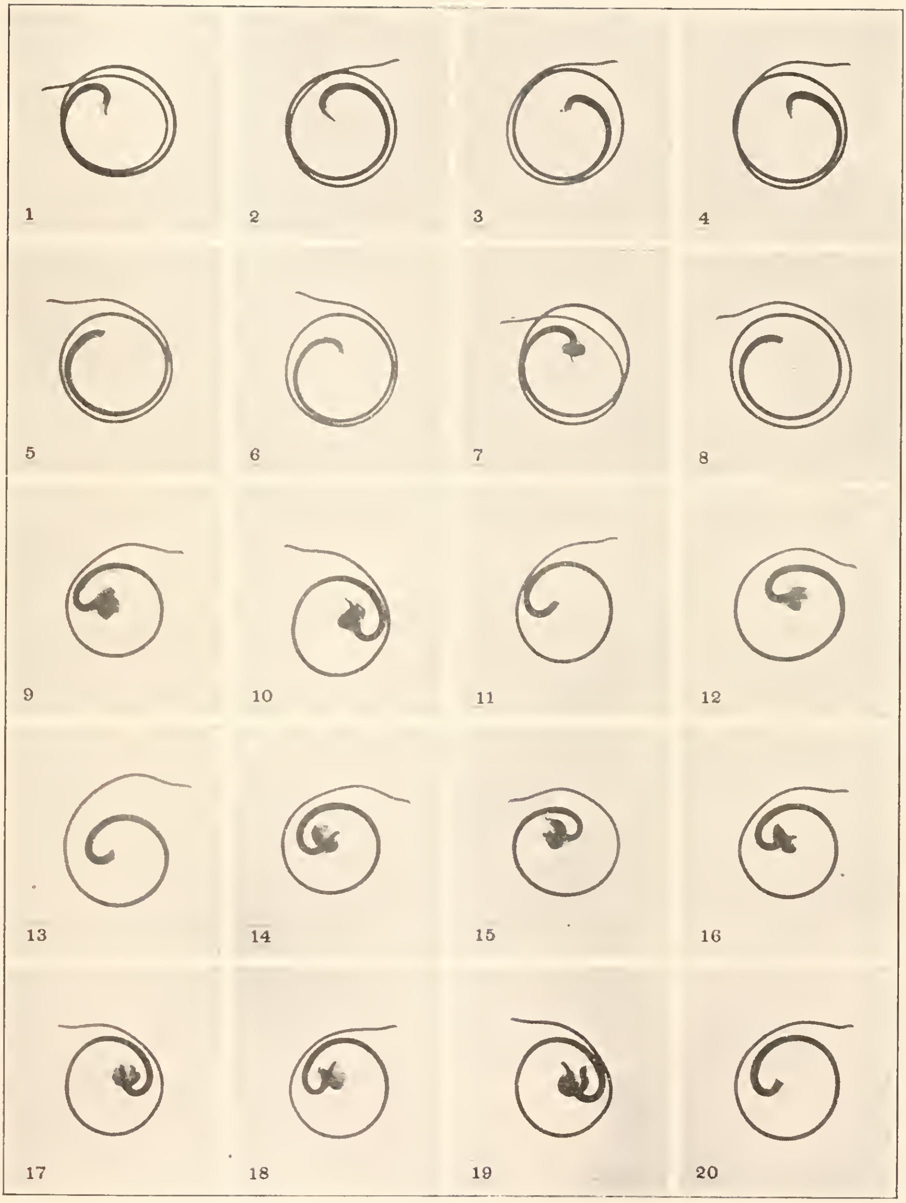
Length of the intromittent organ 60 mm.

Photo 15. Intromittent organ from one of the brothers of the *E. ictericus* that was used for the original cross with *E. variolarius*. These were raised in our laboratory in 1911.

Length of the intromittent organ 54.5 mm.

Photos 16 to 20. Intromittent organs from five typical *E. ictericus* males that were raised in our laboratory during the summer of 1911.

Length of the intromittent organ of photo 16, 58 mm.—of photo 17, 59 mm.—of photo 18, 60 mm.—of photo 19, 61 mm.—of photo 20, 64.5 mm.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K.F. & E.C.S. photo.

INTROMITTENT ORGANS of E. VARIOLARIUS & E. ICTERICUS

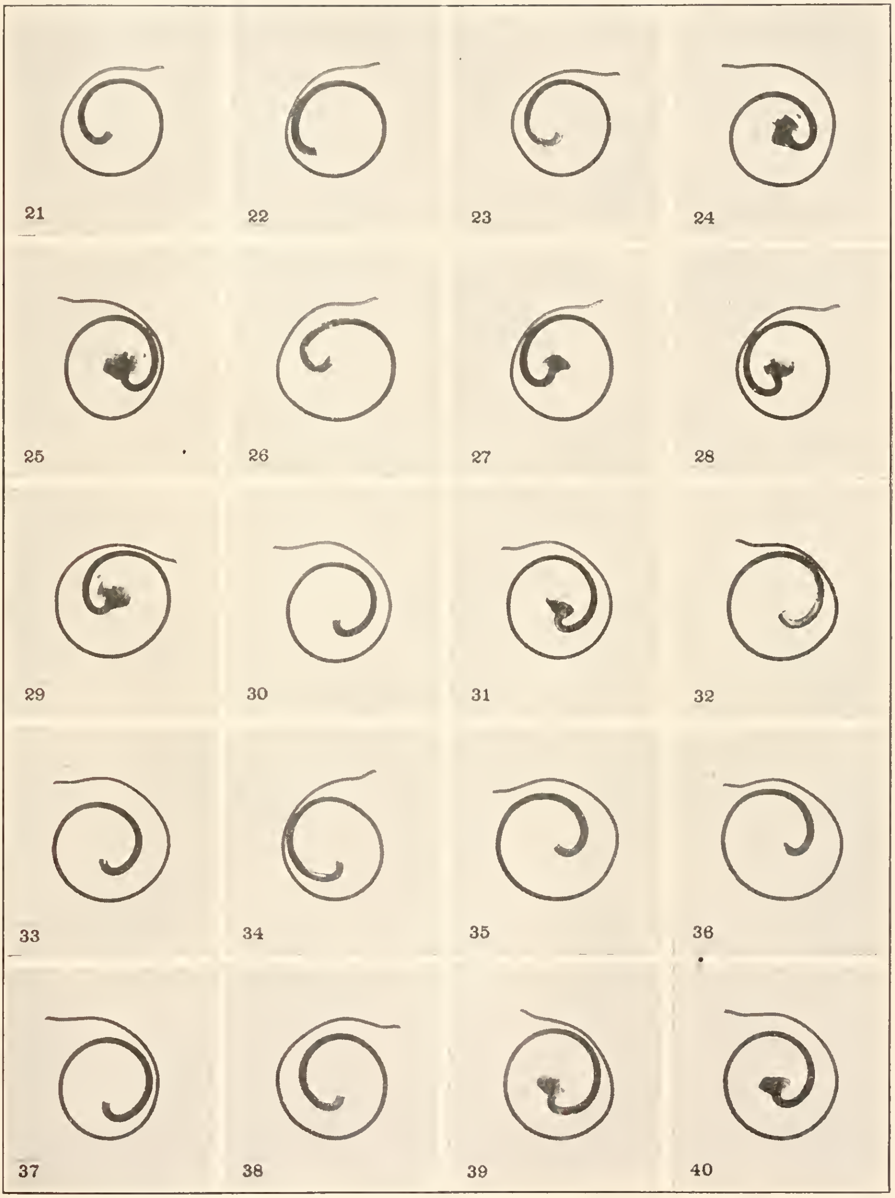
PLATES II AND III (PHOTOS 21–60).

The photographs of these two plates show typical intromittent organs from 40 of the 76 males which we succeeded in rearing from the back-cross with *E. ictericus* male and the F1 female obtained from a cross with *E. variolarius* ♀ × *E. ictericus* ♂.

The photographs of these 40 intromittent organs have been placed on the plates in the order of their size, photo 21 being the shortest (60 mm.) and photo 60 the longest (76.5 mm.).

None of the 71 specimens which were measured can be classed with the *variolarius* intromittent organ which varies in length between 85.5 mm. and 106 mm. Ten of the 71, however, can be classed with the *ictericus* intromittent organ which varies in length between 54.5 mm. and 64.5 mm., though none of the specimens are as short as the shortest *ictericus* organ (54.5 mm.). The remaining 61 are variable intermediates.

The length of the intromittent organs of photos 21 to 60 is as follows: Photo 21, 60 mm.; Photo 22, 60 mm.; Photo 23, 62 mm.; Photo 24, 62 mm.; Photo 25, 62.5 mm.; Photo 26, 63.5 mm.; Photo 27, 64 mm.; Photo 28, 64 mm.; Photo 29, 66 mm.; Photo 30, 66 mm.; Photo 31, 66 mm.; Photo 32, 66.5 mm.; Photo 33, 67 mm.; Photo 34, 68 mm.; Photo 35, 68 mm.; Photo 36, 68 mm.; Photo 37, 68 mm.; Photo 38, 69 mm.; Photo 39, 69 mm.; Photo 40, 69 mm.; Photo 41, 69 mm.; Photo 42, 70 mm.; Photo 43, 70 mm.; Photo 44, 70 mm.; Photo 45, 70 mm.; Photo 46, 71 mm.; Photo 47, 71 mm.; Photo 48, 72 mm.; Photo 49, 72 mm.; Photo 50, 72 mm.; Photo 51, 72 mm.; Photo 52, 72 mm.; Photo 53, 73 mm.; Photo 54, 73.5 mm.; Photo 55, 74 mm.; Photo 56, 74 mm.; Photo 57, 75 mm.; Photo 58, 76 mm.; Photo 59, 76 mm.; Photo 60, 76.5 mm.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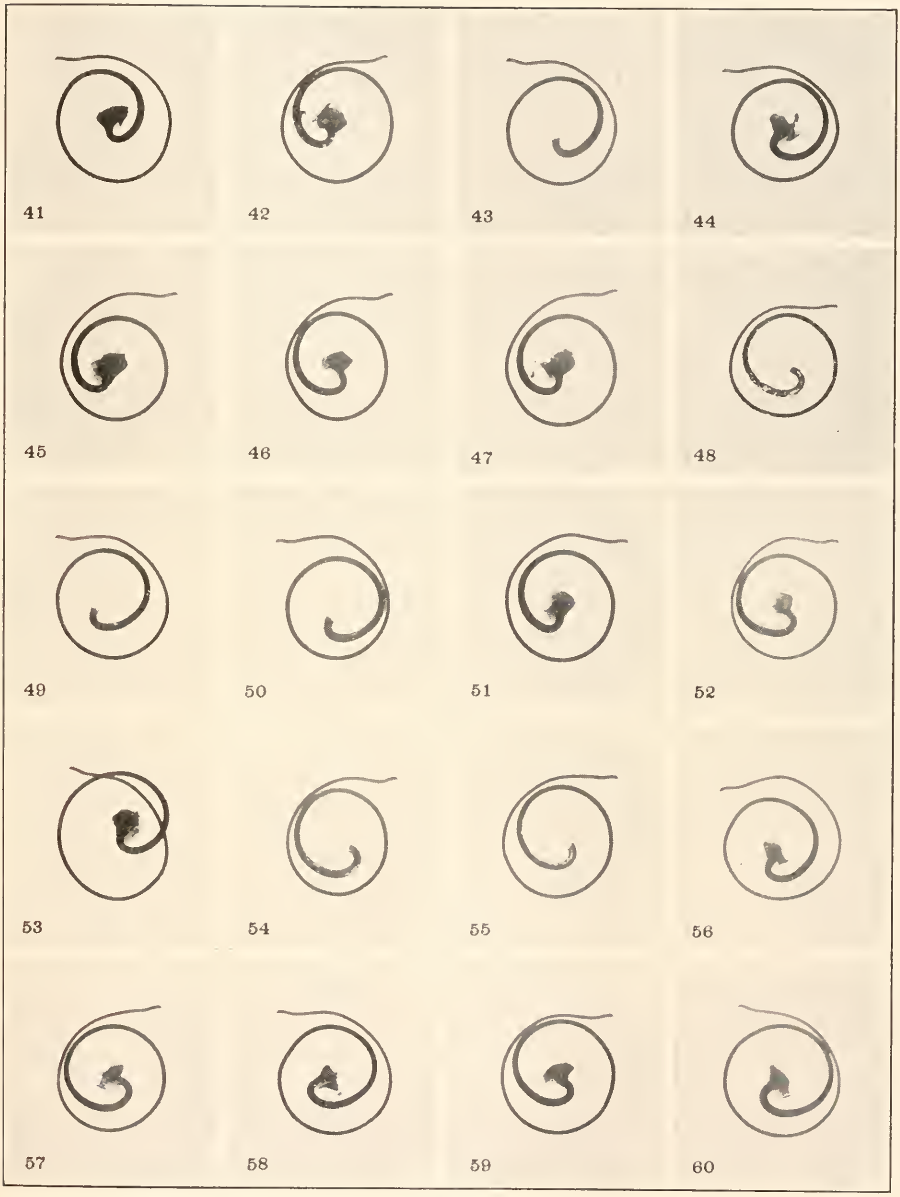
K.F. & E.C.S. photo.

INTROMITTENT ORGANS of the offspring from the BACK-CROSS, F$_1$ ♀ (from E. VARIOLARIUS × E. ICTERICUS) by E. ICTERICUS.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K.F. & E.C.S. photo

INTROMITTENT ORGANS of the offspring from the BACK-CROSS, F_1 ♀ (from E. VARIOLARIUS × E. ICTERICUS) by E. ICTERICUS.

Plate IV.

These photographs of the ventral surface of 41 male insects are of specimens preserved in pure glycerine in test tubes. The genital segment of each insect has been pulled out and cotton inserted behind the segment to hold it in position to show the entire ventral surface.

The magnification is about 1½ diameters.

Photo 61. Seven male specimens of *E. variolarius*, showing the typical black spot on the genital segment. These are the same specimens which were photographed for our report of the cross between *E. variolarius* and *E. servus*.

Photo 62. Seven male specimens of *E. ictericus*, showing that the spot on the genital segment which is so pronounced in *E. variolarius* (photo 61) is only faintly indicated in *E. ictericus*.

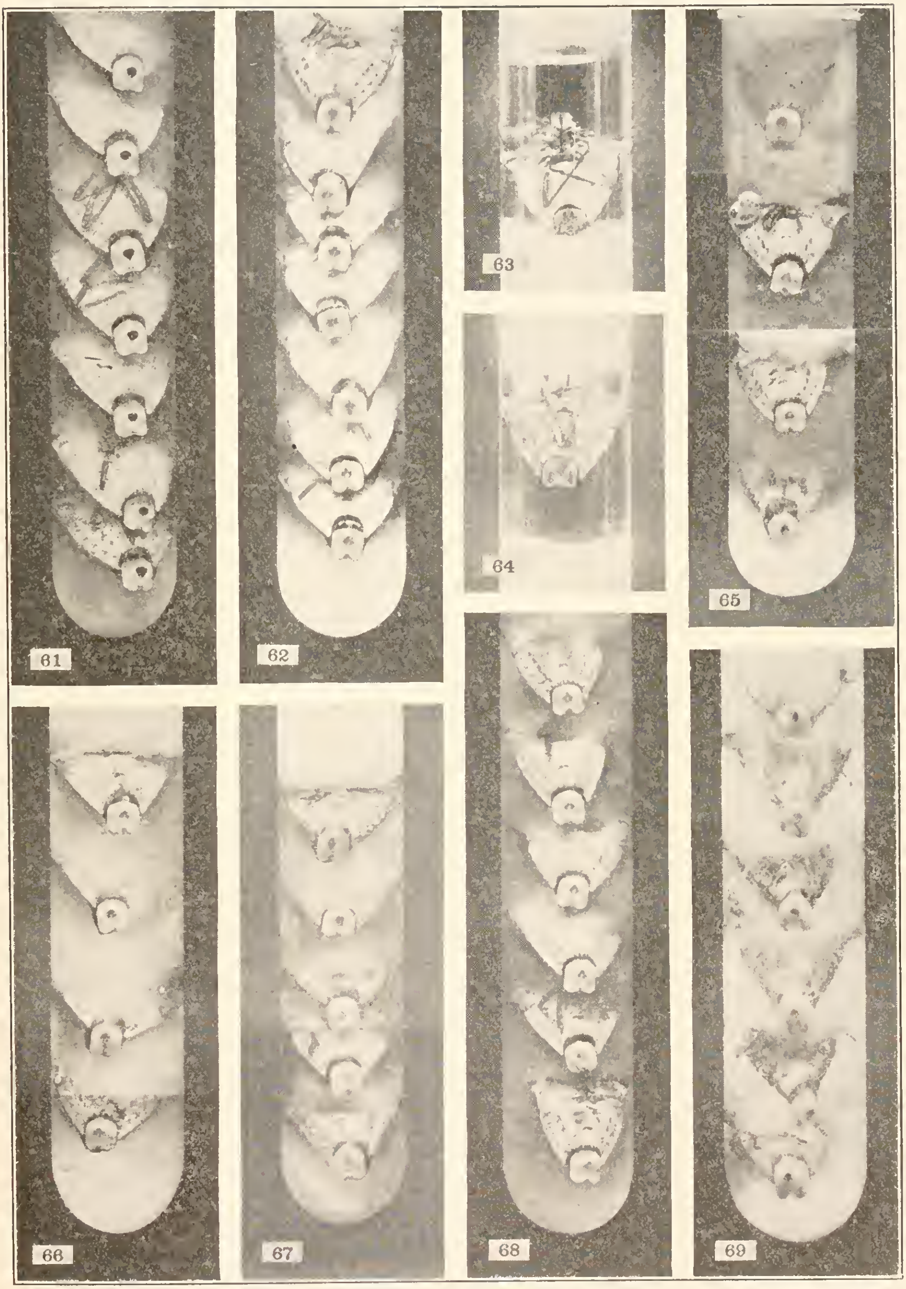
Photo 63. The *E. ictericus* male that fertilized the *E. variolarius* female of the first cross, p. 324. Photo 9 shows the intromittent organ of this insect.

Photo 64. The *ictericus* male that fertilized the F_1 female hybrid that was raised from the cross *E. variolarius* ♀ × *E. ictericus* ♂.[1] From this back cross (see Record, p. 326) 195 eggs were deposited and 177 of these hatched, 149 were reared to the winged stage (76 males and 73 females).

Photos 65 to 69. Twenty-five specimens of the 76 males from the above mentioned back-cross.

A comparison of these 25 specimens with photo 62 shows the influence of the *E. variolarius* grandmother on the genital spot, for a few of these specimens have the genital spot almost as pronounced as that of the pure *variolarius* species and in the majority of the specimens the spot is stronger than that of the pure *ictericus* individuals of photos 62 and 63 and of the pure *ictericus* parent (photo 64). The *ictericus* inheritance is shown in those individuals which have merely a faint indication of the genital spot, *e. g.*, the lower three insects of photo 67 and the lower two of photo 68.

[1] The three apparently pigmented spots on the genital segment of this specimen are misleading,—they are in fact, a slight discoloration of the segment and not a pigmentation. The genital spot of this individual is like that of the lowest specimen of photo 67. Photo 10 shows the intromittent organ of this insect.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MALE SPECIMENS of E. VARIOLARIUS, E. ICTERICUS & offspring from the BACK-CROSS, F_1 ♀ (from E. VARIOLARIUS × E. ICTERICUS) by E. ICTERICUS.

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BIOLOGICAL BULLETIN

A STUDY OF DESICCATION IN THE ROTIFER, PHILODINA ROSEOLA, WITH SPECIAL REFERENCE TO CYTOLOGICAL CHANGES ACCOMPANYING DESICCATION.

LOUIS MAX HICKERNELL.

CONTENTS.

I. Introduction 344
II. Material and Methods 347
III. Observations 350
  1. On Entire Animals 350
    A. Anatomy of *P. roseola* 350
    B. The Active, Free-swimming Animal 353
    C. The Contracted Animal 355
    D. The Dried Animal 356
    E. Animal Recovering from Desiccation 357
  2. Sections 357
    A. The Normal Extended Animal 358
      a. Head Region 358
      b. Esophagus and Salivary Glands 359
      c. Mid-body Region 360
      d. Mid-body Region Containing Egg 360
      e. "Blasendarm" 361
      f. Foot 362
    B. The Contracted Animal 363
      a. Arrangement of Organs in Folded Condition 363
    C. The Dried Animal 363
      a. General Arrangement of Organs 363
      b. Detailed Comparison of Tissues of each Organ with Corresponding Ones in Dried Specimen 364
      c. The Time at which the Rearrangement of Cell Elements Takes Place 368
    D. Animals Recovering from Desiccation 371
      a. General Arrangement and Structure of Organs 372
      b. Detailed Comparison of Tissues of Each Organ with Corresponding Ones in Dried and Undried Specimens 374
IV. Discussion 376
V. Summary 393
VI. Literature Cited 395
VII. Explanation of Figures 398

I. Introduction.

The investigation upon which this report is based was begun while the author was a graduate student in the Department of Biology of Princeton University. A short summary of the earlier findings has been published in a former article ('14). The study of additional material and the completion of the present paper has been carried out in the zoölogical laboratory in the College of Liberal Arts of Syracuse University during the last year. To Dr. E. G. Conklin, who first suggested this investigation to me, I wish to express my thanks for his interest and for the many suggestions made at various times during the course of this study. I am also indebted to Dr. C. W. Hargitt for many favors during the latter part of my work.

The ability of certain rotifers, tardigrades and nematode worms to withstand periods of desiccation has been a subject of investigation for many biologists throughout a period of more than two hundred years. The first recorded observations upon desiccation phenomena are those of von Leeuwenhoek in 1701. From the gutter of a roof he took some dust which he moistened and examined with his microscope. He observed living animals swimming about actively in the water. He found that the animals, which were, no doubt, rotifers, could be deprived of moisture for many months and could then be revived by the addition of water.

After the work of von Leeuwenhoek, the problem of the drying of living things was forgotten for a time. It was not until the period between 1750 and 1775 that interest in the study of desiccation phenomena was revived. During this quarter century many forms were added to the list of animals capable of enduring desiccation.

In 1776 Spallanzani discovered certain tardigrades and nematodes which were able to endure desiccation. He worked also on rotifers and was the first to state that rotifers, when dried free from sand, could not recover from the effects of the drying process. The failure of Spallanzani's rotifers to recover has since been shown to be in no way due to the absence of sand but his experiments aroused much discussion and engaged the attention of many of the foremost naturalists of that day.

Ehrenberg (1838) maintained that the presence of sand protected the rotifers from an actual loss of water. He believed that during the apparent desiccation period all of the vital processes continued, reproduction included. In regard to the function of the sand in retarding desiccation he differed from Spallanzani since the latter believed that the sand protected the rotifer from the injurious effects of the air rather than from loss of water.

Bory St. Vincent, about this time, maintained that under no conditions could the animals survive desiccation but that their apparent revival was due to the hatching of eggs concealed in the sand.

Doyere (1842) after careful study confirmed in the main the observations of Spallanzani. Doyere found, however, that a few rotifers recovered in each lot dried on a clean slide. He disproved the theory of Spallanzani as to the fatal effects of the air, since he found that rotifers dried in air and then placed in a vacuum showed a lower mortality than those dried directly in the vacuum. He concluded that the rapidity of drying is an important factor in the effects of desiccation. He decided from his experiments with rotifers *in vacua* that the last traces of water might be extracted without destroying the power of revival, and that since life processes are impossible in the absence of water, the dried animal possessed life poentially and not actually.

About 1860 a more general interest in the subject of desiccation was revived. The advocates and opponents of the view that desiccation occurs were so evenly divided and the evidence for both views apparently so well founded that the arguments for and against desiccation were considered in 1859 by a commission appointed by the Société de Biologie of France. It was the opinion of this commission that in the dried state life existed only *in potentia*.

Up to this time no one had suggested that rotifers might secrete a water-proof membrane during the dry season. In 1873 Davis said that in *Philodina* there was a secretion of a gelatinous covering which prevented loss of the body fluids. He explained that the presence of sand prevented rapid evaporation of the water and thus gave the rotifers time to secrete the gelatinous envelope before the last traces of moisture on the slide disappeared. This conception has been widely accepted by many up to the present day.

Zacharias (1886) and Faggioli (1891) both reverted to the view of Bory St. Vincent that the supposed revival was due to the hatching of eggs concealed in the sand.

From the time of Faggioli's paper until 1909 no conclusive work on the subject of desiccation appeared. It will at once be seen that the main questions involved in the desiccation problem had not been solved. In 1909 Jacobs, working upon *Philodina roseola* attempted to determine the real conditions attending the drying process.

Jacobs outlines the questions involved in the desiccation problem as follows: "What is the actual effect of drying on a rotifer? Is the water really removed? Does the animal secrete a protecting membrane? If the animal actually dries what is the condition of its tissues? Are life processes at a standstill or are metabolic activities going on all the time in a reduced state?"

Jacobs concluded because of the shrinkage in the tissues, because of negative results in physical and chemical tests for water in the dried animal and as a result of other indirect methods, that the rotifer body becomes truly desiccated. He found no evidence of a waterproof cyst. His answer to the question regarding the condition of the tissues in the dried animal was not very definite, at least not complete, and this for the very good reason that he made no cytological examination of the tissues of the dried rotifers. Jacobs decides that the metabolic changes probably continue in the tissues of dried rotifers.

In all the discussion and observation upon the subject of desiccation phenomena previous to the publication of the preliminary note on this work, apparently no one had considered the problem from a cytological point of view. This is true not only of the study of animal tissues, but among plants as well. Certain algæ, pteridophytes and liverworts can be dried and will subsequently recover. This fact has been known for years. Pfeffer (1903) comments upon physiological phenomena attending desiccation but mentions no changes in structure. The author published in 1914 a preliminary account of the observations recorded in this paper. Brown (1915) working with McDougal at the

Desert Laboratory at Tucson, Ariz., describes cytological changes accompanying desiccation and subsequent recovery in the cells of *Echinocactus wislizeni*. These two papers, as far as is known to the author, comprise the literature on the cytology of desiccation.

Although this paper deals with the same kind of material as was used by Jacobs and although some of the questions asked by him are of most interest here, the problem has been attacked from an entirely different angle and by cytological methods confirms and adds to the results of Jacobs which were obtained by physiological methods.

The purpose of this study is, therefore, to attempt by means of cytological examination to determine (1) the condition of the tissues of the dried rotifer; (2) the presence or absence of a protecting membrane in the dried condition; (3) the condition of metabolic activity in the tissues of the dried animal and (4) the changes attending a recovery from desiccation. In order to do this it will be necessary to compare carefully the cytological appearance of sections of animals in a condition of dryness and those of animals recovering from desiccation with sections of the normal, active animal. From the data so gathered the conclusions will be drawn.

II. Material and Methods.

Philodina roseola is one of the common rotifers belonging to the order Bdelloideæ. It occurs throughout the world and is found in depressions in rocks, eave troughs or almost any place which is moist or periodically moist and dry. It is usually associated with the unicellular alga, *Sphærella lacustris*. Frequently one notices small individuals in hay infusions. These are, no doubt, introduced when dry hay is added to old cultures. Sometimes in infusions rotifers are found which are fully as large as those associated with the alga before mentioned. These may be encouraged to multiply if very weak hay infusion is added from time to time. The weak infusion allows sufficient bacteria to develop to replace the algæ as food and it seems not to harm the animals.

As has been stated by other authors, the behavior of *P. roseola* is dependent upon a number of factors, chief of which seem to be the purity of the medium in which it lives, the amount of food material present and the temperature of the medium. If the water surrounding the animals does not contain too much organic material, they usually may be seen stretched out, fastened by the foot and going through the usual feeding movements. When conditions are unfavorable they contract (Pl. II., Fig. 4) and remain quiet until the surroundings become more favorable. A slight increase in temperature is usually conducive to more vigorous movements, especially swimming.

In preparing *P. roseola* for microscopic examination or subsequent sectioning some difficulty may be experienced because of the fact that the animals are generally associated with sand or other foreign material. When the animals are present in sufficient numbers the method of collection suggested by Jacobs is perhaps the best. This takes advantage of the reaction of the animals to light. If subjected to no mechanical stimulus they usually are indifferent to natural light of ordinary intensity. However, if the culture is violently agitated by shaking or stirring they become temporarily negatively phototropic and may be collected with a pipette from the darker part of the culture. When it was desirable to select individuals of any given size or condition of maturity in these experiments, or to get some absolutely free from foreign material this was accomplished by picking up the animals one by one from a Syracuse watch glass with a capillary pipette, the entire process being watched with a binocular microscope. The foregoing statement is not to be interpreted as meaning that *P. roseola* is ever covered with debris. Such is not the case. The foreign material alluded to consists of bits of sand, etc., to which the animals adhere by means of the sticky secretion of the foot glands.

When first put on the slide under a cover glass the animals are generally so active that a single individual can be kept within the field of a high power lens only with greatest difficulty. As some of the water evaporates from under the cover glass, and as the latter gently presses upon the animal, movements become less rapid and many details of organization can be made out with little trouble.

By putting the rotifer in a weak solution of neutral red some of the internal organs are stained but not with any particular sharpness. By proper manipulation of the light through the condenser the unstained specimen becomes practically as favorable for examination as that stained in neutral red.

In preparing dried animals for sectioning large numbers of active animals, free from sand, were put in Syracuse watch glasses and the latter placed in an oven at about 40 degrees Centigrade. While the desiccation process is always fatal to a few animals in each lot which is being dried, I can affirm what Jacobs has already pointed out, namely, that in the case of animals dried on clean glass the mortality is lowest when the temperature is about 40 degrees Centigrade and the moisture is not allowed to pass off too rapidly.

In preparing undried rotifers for sectioning a large number was collected in a test tube and anesthetized with the cocaine-methyl-alcohol mixture of Rousellet. When the animals were completely relaxed the excess liquid was drawn off and the fixing fluid poured upon the entire mass. After fixation the material was washed thoroughly, passed rapidly through the alcohols, cleared in xylol and embedded in paraffine of 54–56 degrees melting point. Sections were cut 3–5 μ in thickness.

In the case of the dried animals the fixative was poured directly upon them and allowed to act from twenty to thirty minutes.

Several fixing fluids were employed. Bouin's picro-acetic formol was not very satisfactory. A solution of saturated corrosive sublimate with 5 per cent. acetic acid was used hot. This was quite satisfactory in that its action was almost instantaneous and the preservation of structures faithful in most respects. Beauchamp ('09) says that sublimate fixation was very poor in the forms he studied. He does not state the exact formula employed in his experiments but it would appear that he did not try the one mentioned above. By far the best formula for fixation is the osmic-sublimate-bichromate mixture recommended by Beauchamp.

In staining the sections iron-alum hæmatoxylin combined with various counter stains gave the best results. The iron-hæmatoxylin-eosin-lichtgrün stain of Beauchamp is very valuable. Another combination consisting of safranin, orange G and lichtgrün was used with some success. The latter is not so precise as the hæmatoxylin combination.

OBSERVATIONS. 1. *On Entire Animals.*

A. *Anatomy of P. roseola.*—Janson ('93) has carefully considered the anatomy of the Philodinidæ and I find few inaccuracies in his statements as applied to *P. roseola*. It will be necessary, however, to briefly describe the organ systems and their relationships in order to arrive at a thorough understanding of the changes in form and rearrangement of parts incident to the desiccation process.

The body of *P. roseola*, at first sight, suggests that of a segmented worm. Its segmentation (which affects only the integument and accessory structures) is, however, not so regular but that the body is easily divided into head, trunk and foot. Its outer integument is formed of a thin, more or less transparent cuticle beneath which is a granular plasma layer, the hypodermis. In the hypodermis are deposited pigment granules. These are absent in the very young animal and occur in varying number in the adult. The cuticle is soft and flexible, easily lending itself to the telescoping and folding so characteristic of this *Philodina*.

The hypodermis is a syncytium which contains nuclei distributed evenly through it. There are, in the mid-body region, longitudinal folds of the skin which are always present. Temporary cross foldings occur as a result of the vigorous movements of the animal. Externally the skin lacks spines or protuberances of any kind.

The head, whose most prominent structures are the trochal discs, extends as far back as the forward margin of the mastax. When the trochal discs are folded in, a blunt, ciliated projection is seen at the anterior end. This is the proboscis and it is used for tactile purposes as well as to aid in the "measuring-worm" type of locomotion so characteristic of the Bdelloideæ. The rotating organs consist of a double row of cilia, broken in the case of the anterior row, in the ventral part of the head. The more posterior ciliary wreath is made up of shorter elements and is directly continuous with the cilia which line the pharangeal cavity. The hypodermis in the head region at the base of the trochal discs is thickened, probably to furnish additional support for the rotating apparatus, and in some specimens the nuclei of this layer can be seen near the bases of the lines of cilia.

Dorsally, and arising at the posterior margin of the head, is the tactile organ (Pl. I., Fig. 1, *t. o.*) which shows from two to four segments according to its state of extension. It is capped at the end by a tuft of short cilia. Nerve fibers connect it with the brain as Zelinka has shown.

The brain (Fig. 1, *br.*) lies with its anterior margin just at the base of the tactile organ, while it extends posteriorly slightly over the forward part of the mastax. It is triangular in shape with the sharpest angle extending forward. The eyes (Fig. 1, *e*) which are red in color, lie dorsal to the brain.

The excretory canals (Fig. 2, *e. c.*) extend into the head and a terminal flame cell (*f. c.*) can sometimes be seen beating at the base of each trochal disc. These flame cells are perhaps best seen when the trochal discs are folded in. They appear very close to the margins of the folded discs (Pl. II., Fig. 3).

The trunk is almost entirely filled with the organs of digestion and those of reproduction. In its most anterior part the mastax (Pl. I., Fig. 1, *m*) is prominent. Food enters this by way of the narrow, ciliated pharanyx (Pl. I., Fig. 2, *ph.*). The mastax proper consists of two more or less crescent-shaped jaws. Transverse ridges, which at the inner margins continue as short projections, form the teeth (Pl. I., Fig. 1, *t*). In all the animals I have studied there have been two teeth on each jaw. A few authors report cases where there are three teeth on one jaw and two on the other.

The mastax is surrounded by glands which probably contribute a digestive fluid to the food as it is being crushed in the mastax. There are five of these salivary glands, two of which lie dorsally and three ventrally. The dorsal ones (Fig. 1, *s. g.*) follow the outline of the mastax quite closely, while those on the ventral side are usually larger and extend posteriorly (Fig. 2, *s. g.*). In the living animal these glands show a granular cytoplasm with nuclei of varying sizes. These nuclei appear as bright spots against the darker cytoplasmic background.

From the mastax a narrow esophagus leads to the stomach-intestine (Pl. I., Fig. 1, *st. i.*). In most cases it is difficult to see the esophagus in the living animal. This is also true of a pair of glands which lie at the anterior end of the stomach-intestine. Both the esophagus and these glands are obscured by the ventrally lying salivary glands, and it is only when the animal is thoroughly stretched that these structures can be distinguished.

The stomach-intestine, as seen in the living animal, consists of a yellow, viscous tissue containing granules, fat-droplets and nuclei. By careful observation all of these elements may be recognized. The lumen is usually not seen except in sections. In healthy, active animals the stomach tissue shows its color distinctly. In starved animals the color is lacking in the stomach tissue as Zelinka has pointed out. In newly hatched *Philodina* the stomach is almost perfectly transparent.

Just posterior to the stomach-intestine is the "blasendarm" (Fig. 1, *bl.*) of Zelinka. This is thick-walled but not glandular. It serves for the accumulation of the undigested remains of the food. By the contractions of its walls this material is evacuated from time to time. Darker in color and more homogeneous in structure, this first division of the end gut is the most prominent structure in the posterior part of the trunk of the living *Philodina*.

The "blasendarm" leads into the rectum (Fig. 1, *r*), a narrower tube in the dorsal wall of which the contractile bladder (Fig. 1, *c. b.*) is found. This bladder is thin-walled, extensible and on either side receives the lateral excretory canals. The bladder pulsates at intervals of fifteen to twenty seconds and in so doing disposes of the accumulated waste which has been emptied into it by the excretory canals.

Lying on either side of the stomach-intestine are the reproductive organs. Since no males of *Philodina* are known, these organs are always ovaries with their accessory structures. The ovary consists of from six to ten small nuclei which lie close together in a clear syncytial ground substance on the inner margin of the vitellarium. It is not easily seen in living animals unless it contains a developing egg (Pl. I., Fig. 1, *eg.*).

The vitellarium (Fig. 1, *v*) is especially prominent in living animals. It is a spindle-shaped structure at either end of which is a connective tissue strand which fastens it to the other organs. The anterior strand is fastened to the body wall in the region of the mastax, the posterior one attaches to the digestive system in the region of the boundary between the "blasendarm" and contractile bladder. The posterior strand looks as if it might act as an oviduct but I have never seen an egg or an embryo passing through it. This fact, together with observations of other authors who have seen embryos of *Philodina* liberated from the mother by a rupture of the body wall, makes it seem certain that if this structure ever did function as an oviduct it is now only rudimentary. The vitellarium contains eight large nuclei arranged in a row. This number may vary but this is not usually the case.

The lateral excretory canals run posteriorly through the trunk and very close to the vitellaria. They are minute, thread-like structures having flame cells connected to them at intervals. In *P. roseola* I have never observed more than five of these flame cells on each side. The canals empty into the contractile bladder as was described above.

The rectum is a narrow tube leading posteriorly from the contractile bladder and terminating dorsally in the anus (Fig. 1, *an.*) at the posterior border of the second foot segment.

The foot contains the glands which secrete a sticky substance which enables the animal to fix itself during its crawling movements. These glands are oval in outline, are uninuclear and empty by means of ducts at the base of the toes. When the animal is folded up these glands become packed closely about the "blasendarm." When the rotifer stretches out they occupy places in the four posterior foot segments. There are four toes on the tip of the foot. These aid in fixing the animal during feeding movements.

B. *The Active, Free-swimming Animal.*—*P. roseola* in its behavior shows no great difference from most of the other Philodinidæ. Zelinka ('86) and Janson ('93) have described the movements of the philodinids in general terms while Jacobs ('09) has given an account of *P. roseola* both under ordinary conditions and at the onset of desiccation. I have given the behavior of *P. roseola* much attention during the course of this work and am able to confirm Jacobs in all essential respects.

Two methods of locomotion are employed, viz., swimming by means of the trochal cilia and creeping by the alternate use of foot and proboscis. The method of locomotion depends to a large extent upon the temperature and purity of water in which the animal lives. Other things being equal, a relatively high temperature is conducive to active swimming movements, while with lower temperatures the animal generally restricts itself to the leech-like creeping. Another factor influencing the kind of movement is the purity of the water. In cultures which contain many putrefactive bacteria the animals usually are found creeping sluggishly about, and they seldom extend the trochal cilia for any great length of time.

Swimming is the result of the rotating of the trochal cilia and involves no turning upon the body axis. The body movements during swimming are similar to those of certain infusoria as described by Jennings ('04). If any obstruction is met with in swimming the trochal cilia are suddenly retracted and a series of random testing movements occur. The cilia are then extended and swimming is resumed in a direction where there is no obstruction. Sometimes an animal will be observed to stop if it swims through a region where food is abundant. In this case it attaches itself by the foot and feeding movements begin.

In creeping the rotating organs are always retracted. The animal attaches itself by means of the secretion of the pedal glands and then after a greater or less number of testing movements, in which the body is stretched successively in several directions, it attaches itself by the end of the proboscis. This attachment of the head region is followed by the wrenching loose of the foot. The latter is then brought to a point nearer the head and the operation is complete. In changing from the creeping to the swimming movement the trochal discs are extended and the foot is loosened by a contraction of the muscles in the posterior region. The animal then moves steadily forward as long as its trochal cilia are in motion.

While feeding the animal is always attached by the foot. Currents created by the rotating organs carry bacteria, algæ, etc., in a steady stream into the open pharynx where they are further propelled by the long cilia lining the pharynx. The body may sway from side to side during feeding, in fact, the animal is frequently seen to bend its body as if to reach some particles which were not before influenced by its trochal activity. During all this time the mastax is active. Its grinding operations are easily observed in any feeding animal.

C. *The Contracted Animal.*—Zelinka ('86) in describing the movements of rotifers of the genus *Callidina* states that the animals frequently retract the head and rotating organs without any apparent reason. He observes further that their subsequent extension may be rapid or slow and that no definite reason can be assigned for the lack of uniformity in this respect among different individuals. *P. roseola* contracts very rapidly but usually extends itself slowly. The factors which influence it to contract seem to be (1) Sudden change in temperature of the medium; (2) change in the chemical composition of the medium; (3) mechanical stimuli; and (4) desiccation.

Gradual changes in temperature in the medium in which the *Philodina* lives do not cause it to contract. Increase in temperature is conducive to more vigorous movements while a decrease causes the animal to become sluggish. If the temperature is suddenly changed by the addition of hot or cold water the animal contracts and does not extend itself for many minutes.

The addition of any active chemical to the culture causes the rotifers to suddenly contract and if the foreign substance is not removed they will remain in the contracted condition until death ensues. Philodinas kept in infusions will frequently be found to become inactive in this way. An examination usually shows that the infusion is in too great concentration or that putrefactive or acid forming bacteria have rendered the environment unfavorable.

A mechanical shock of any kind will make the animals contract completely but a contraction under such conditions usually lasts but a few seconds unless the stimulus is continuous or repeated.

When the water around a *Philodina* begins to dry up the animal creeps about rapidly apparently trying to escape from the diminishing drop. This creeping continues until movement is no longer possible. The animal then contracts into an almost spherical mass and dries.

Especially interesting to observe is the manner in which the organs are arranged in the contracted animal. While in the extended animal there are spaces between organs, when contraction takes place every available bit of space inside the trunk integument is filled by the closely packed organs. The head and foot segments are drawn entirely within the trunk by means of the contraction of longitudinal muscles. The circular muscles at each end of the trunk may then contract and cause the entire animal to assume a shape not unlike a lemon. The large organ systems can be seen through the integument of the contracted rotifer. The head and its accessory structures can be seen just anterior to the stomach tissue. The stomach occupies the central part of the mass and is flanked on either side by the reproductive organs. In the posterior end of the trunk the foot segments containing the pedal glands can be seen. Fig. 4, Plate II., shows a *Philodina* which has been contracted but is just beginning to unfold. The foot segments are slightly protruded while the head still remains within the trunk cavity.

D. *The Dried Animal.*—When the drop of water surrounding a *Philodina* begins to dry up the animal indulges in active creeping movements until the drop becomes too small to permit further activity. The rotifer then contracts and actual desiccation begins. It is at this time, according to the older authors, that the jelly membrane is secreted. The shiny appearance described by them is indeed apparent at times but this is due not to any jelly but to the fact that the tissues of the rotifers become packed together as water disappears and since loss of transparency accompanies loss of water the light is reflected rather than transmitted by the animal. Final proof of the absence of a jelly layer in dried rotifers will be given in the part dealing with sections.

The internal organs cannot be carefully studied while the drying is going on since the integument usually folds and wrinkles as soon as drying begins. This obscures the internal structures.

Animals dried under favorable conditions all tend to assume a similar shape. The head and foot segments are drawn into the trunk as before described. The circular muscles at the end of the trunk contract giving to the animal a spindle shape. When the drying process is very slow the irregular wrinkles do not appear but the puckering due to the contraction of the circular muscles is evident. Jacobs has pointed out this fact and my Fig. 16, a longitudinal section through a dried animal, shows the sides as being free from folds while the ends show prominent and regular wrinkles.

Frequently several animals are carried together by the diminishing drop as it evaporates. In this case each rotifer tends to assume a hexagonal shape as a result of mutual pressure. Fig. 5, Plate II., shows an animal which was dried on a clean slide at room temperature and drawn nine days after the desiccation began. It will be seen at once that the integument has become much folded and the internal organs indistinct, but the vitellarium, foot and stomach are still recognizable.

E. *Animals Recovering from Desiccation.*—When water is added to dried animals, such as that described in the last section, they usually regain their normal size within ten minutes, often before. The rapid swelling of the animal causes any wrinkles which may be in the integument to disappear. Active movements may occur within a short time if the conditions under which they are dried are favorable. Sometimes movements may not occur for several hours, even a whole day. This fact may account for the negative results of the desiccation experiments of some of the older authors. These investigators probably did not give their rotifers sufficient time to recover, as Jacobs points out.

As the animals recover they resume their usual activities. I have noticed that rotifers recovering from desiccation are lighter in color than they were before the process began. Further comment will be made upon this point in a later paragraph.

2. *Study of Sections.*

The cytological details of the structure of rotifers has been described in comparatively few cases. Of the Bdelloideæ, *Discopus synaptæ* has been described by Zelinka ('86). From the study of the figures of sections through the principal body regions as represented by Zelinka one can see many points of similarity between this form and *P. roseola*. In the case of *P. roseola* I find no record in the literature which tells of the cytological structure of its tissues. The accompanying figures and descriptions are therefore presented here for the first time.

The finer cytological changes accompanying the process of desiccation and subsequent recovery can only be shown after describing the conditions present in a normal, undried specimen. I shall therefore now describe the arrangement of organs and their cytological peculiarities in both extended and contracted normal animals, after which comparisons will be made with sections of animals which have been desiccated and also with sections of animals recovering from desiccation.

A. *The Normal Extended Animal.*—The relationship of the internal organs is well shown in transverse sections of the extended *Philodina*. Fig. 7, Plate III., shows a section through the posterior part of the head. The pharyngeal cavity leading to the mastax is lined with cilia. Its wall consists of thick cells with large nuclei. The brain, with outer cellular elements and inner homogeneous zone, appears close to the pharyngeal tube. The integument surrounding the region of this section is much thinner than it is in the mid-body region. This is to be expected when one remembers the flexibility of the head segments as compared with those of the trunk. There is a marked similarity in the arrangement of the elements here described and those in a transverse section through the anterior part of *Discopus synaptæ* as described by Zelinka ('88).

In *Discopus*, Zelinka shows the integument as being much thinner than I have found it in *Philodina*. The hypodermis of *Philodina* appears as a definite layer much thicker than the cuticle and containing nuclei embedded in its syncytial groundwork. This feature is seen in the figures of the sections throughout all parts of the animal's body. In *Discopus* the nuclei are flattened rather than round and appear as swellings upon the cuticle. Furthermore, Zelinka figures the hypodermal nuclei as being without a nucleolus. Such a condition is not usually found in *Philodina*. Another point of variance between the two forms concerns the brain. Zelinka figures the brain of *Discopus* as being composed of an outer ganglionic layer and a central "punktsubstanz." In *Philodina* the same divisions appear but the cells of the ganglionic layer have distinct walls as distinguished from the syncytial condition in the brain of *Discopus*. The walls of the pharynx with their internal ciliation are not markedly different in the two forms.

A section through the esophagus is shown in Fig. 8, Plate III. As was mentioned before, the esophagus is difficult to observe in the living animal because it is generally concealed by the salivary glands which surround it. The section shows it to be a narrow, thin-walled tube. Surrounding it are the salivary glands whose cytoplasm is uniformly granular but not divided into distinct cells. The nuclei appear scattered irregularly and have the characteristic structure found in like elements of other tissues.

The esophagus in section in *Discopus* shows about the same relative size and position as it does in *Philodina*. It is likewise surrounded by the salivary glands so that it probably is not visible in the living animal. The salivary glands of *Discopus* do not appear to be as dense or deeply staining as those of *Philodina*. The cytoplasmic granules are less closely packed together and the nuclei appear to be less chromatic. The most noticeable difference between the figures of sections of corresponding regions in the two animals is the lack of hypodermal tissue in *Discopus*. While it is to be expected that the integumentary structures would be thinnest at the points of greatest flexibility, it seems that the total absence of hypodermis in this and some other of Zelinka's figures must have been a result of an oversight on his part.

A section through the middle portion of the trunk (Fig. 9, Plate III.) shows the reproductive glands and the middle portion of the digestive system surrounded by a somewhat thicker ring of the integument. The plasma of the vitellarium is a syncytium made up of granules of varying sizes. The granules are surrounded by small, clear areas containing a cell-sap which is lost during desiccation. The nuclei of the vitellaria (*v*) are the largest to be found in the rotifer body. These consist of a single karyosome surrounded by a clear homogeneous area and having at the periphery a distinct nuclear membrane. This is the "nucleolar nucleus" of Carnoy and is characteristic of most of the cells of the rotifer tissues. The ovary (*ov*) is a small elongated structure lying in a depression of the inner border of the vitellarium. It never attains very great size. It consists of from five to twelve intensely chromatic nuclei surrounded by a glassy, homogeneous plasma. A section of a typical ovary is shown in Fig. 10, Plate III. Both ovary and vitellarium are surrounded by a delicate membrane.

Zelinka does not figure a section of the ovary of *Discopus* containing more than four nuclei. This small number is frequently observed in *Philodina* but the average number is slightly larger. The clear, non-staining cytoplasmic portion of the ovary seems to be similar in both forms but Zelinka does not figure a membrane separating ovary and vitellarium. Whether this last point was due to improper or insufficient staining is hard to say but it seems that a limiting membrane should be present. In *Philodina* a definite ovarian capsule can be easily demonstrated in sections through the proper region of the normal, free-swimming animals.

Another point which leads me to believe that the staining methods employed by Zelinka were insufficient to demonstrate all structural details is the fact that he does not figure a nuclear membrane in the nuclei of the vitellarium. The karyosome is surrounded by a clear area as in *Philodina* but he figures no limiting membrane about the entire structure. It is possible that he thought the karyosome to represent the entire nucleus. However this may be, there is, in all cases, in the vitellarium of *Philodina* a definite nuclear membrane surrounding the clear, outer zone of the nucleus.

In cross-section the stomach-intestine (Plate III., Fig. 9, *st*) shows as a thick-walled tube with a narrow lumen. From the inner wall cilia project into the lumen while at the base of the cilia are deeply staining granules, which Beauchamp, in other rotifers, interprets as cross-sections of longitudinal muscle fibers. My own observations lead me to believe that this interpretation is correct. The stomach tissue is of a spongy consistency, the ground substance appearing as closely packed vacuoles. No cell walls can be demonstrated but nuclei are scattered at intervals throughout its extent. The nucleus has a karyosome surrounded by a homogeneous plasma which stains with acid dyes. Scattered irregularly through the stomach tissue are large, deeply staining granules which are masses of reserve food material. Janson ('93) says that these masses are kept by the rotifer for use during the dry periods when feeding must of necessity cease. Beauchamp ('09) shows sections of the stomach of *Hydatina senta* in which these granules appear. He shows further that in a section of the stomach of an individual of the same species which had been starved for twenty-two days, these granules had disappeared and were replaced by vacuoles. In sections of very young *Philodinas* no such granules are present and I shall show later that after periods of desiccation in *Philodina* these granules become fewer or disappear entirely, thus furnishing cytological evidence in support of the theory of Janson and Zelinka, which by them was based entirely upon observations upon the entire, living rotifers.

The stomach tissue is most pliable and the lumen seems normally to be able to occupy almost any position in it. This fact has been remarked by Beauchamp in *Callidina socialis*.

The stomach tissue of *Discopus* as figured by Zelinka lacks some of the elements which I find in *Philodina*. I refer to the granules of reserve food material just mentioned. In none of his figures does he show these deeply staining aggregations. It may be that the digestive processes of *Discopus* differ from those of *Philodina*. The difference in the habits of the two forms might account for this. On the other hand these elements might have been present but not differentiated by the stain. The latter condition does not seem probable, however, for I have found that most of the nuclear stains have an affinity for the food granules.

Another point of difference is in the structure of the ground substance of the stomach tissue. In *Discopus* this ground substance is represented by Zelinka as being made up of fine granules loosely packed together. In *Philodina* the ground substance of the stomach tissue is composed of alveoles closely apposed. This difference could perhaps be accounted for upon the basis of fixation.

Fig. 11, Plate III., shows a section through the most posterior part of the trunk at the point where the stomach-intestine joins the "blasendarm." The contrast in the structure of the two tissues is well marked. The "blasendarm" contains none of the reserve food granules and its plasma has only occasional nuclei scattered through it. Zelinka does not show a section through this region of *Discopus* but there is probably no great difference here between the two animals.

Fig. 12, Plate III., is a transverse section through the second foot segment. It shows the pedal gland (*f. g.*) whose cytoplasm consists of fine alveoles and whose cell-walls are distinct. A single nucleus is present in each cell. The form of this nucleus is subject to little variation in the glands of the undried animal. It consists of a karyosome surrounded by a clear space and a well defined nuclear membrane.

The foot-glands of *Discopus* differ but slightly from those just described. The individual cells are perhaps more nearly round than those of *Philodina* and the chromatic part of the nucleus more nearly spherical. The general arrangement and appearance of these cells is, however, not much different from those of *Philodina*.

Ventral to the pedal glands and separated from them by a narrow space, lies the cloacal cavity. This section throws some light upon the nature of the contractile bladder itself. It has been assumed by Huxley, Claus, Vogt and Yung, Hudson and Gosse, that the contractile bladder is merely the enlarged ending of the lateral canals and must therefore be considered as a part of the excretory system in a strict sense. Semper thought that the contractile bladder merely forced the excretory fluid into the end-gut and that other contractions of the cloacal wall were necessary before the fluid reached the exterior.

In Fig. 12 the cloacal chamber is shown to be composed dorsally of a thin membrane such as one would expect in a structure as distensible as the contractile bladder is known to be. Ventrally the wall is thicker and in all respects similar in its texture to that of the "blasendarm" (Fig. 11). On one side and at the junction of the thin dorsal and thick ventral walls there is a break which I interpret as the entrance of the excretory canal. The large cell lying beside the entrance of this canal is part of the sphincter which prevents the fluid from re-entering the lateral canals at the time of the contractions of the bladder.

This sphincter has been described by other authors but sections of it in *Philodina* have never been shown.

It is evident then, that the contractile bladder is not an independent structure connected to the posterior part of the gut by a narrow neck but is only a portion of the wall of the gut modified to permit its distension. Its periodic contractions force the collected liquid waste to the exterior without further contractions of other parts of the cloaca. The more solid waste from the digestive canal is forced out of the "blasendarm" by independent contractions of the latter structure.

B. *The Contracted Animal.*—The closely packed condition of the organs in the folded animal is shown in Fig. 13, Plate IV. The cilia of the trochal discs and some of those lining the pharynx will be noticed. On either side of the infolded trochal cilia are the ends of the large glands connected with the mastax and with the anterior end of the stomach-intestine. The edge of the chewing apparatus is shown in the middle of the section, while surrounding it will be seen the stomach tissue and a small part of its lumen. The folding of the integument of the animal is well shown here, especially at the anterior end where the convergence of the longitudinal folds shows in section as a rosette. It is apparent that in the contracted condition most of the water which circulates in the body spaces is lost, for the organs lie close to each other in contrast to the condition shown in Figs. 1, 2 and 3 where the spaces, especially those between the integument and internal organs, are very large. Fig. 14, Plate IV., shows a section through a slightly different plane in an undried, contracted animal. In the central part of the section the cells of the outer part of the brain are shown while around the periphery various gland cells appear. This section also goes through one of the vitellaria and shows its characteristic form and structure.

C. *The Dried Animal.*—In the desiccated animal the arrangement of organs is in no way different from that in the previously described contracted, normal ones. The organs are still more closely apposed and the folding of the integument is closer and better marked.

Fig. 16, Plate IV., shows a section of a rotifer which was dried at room temperature and then kept in an evacuated calcium chloride desiccator for eighteen days. At the end of this time it was fixed and sectioned. It will be observed that the cilia still show as individual fibers and have not fused into a homogeneous mass as some authors assert must be the case when all moisture is removed. There is no sign of fusion or other abnormal condition in these elements. Each cilium preserves its identity as well as would those of an animal living in its natural environment.

Of especial interest is the structure of the integument in the dried state. It has been asserted, and for some time quite generally believed, that just before actual drying takes place the rotifer secretes a jelly-like capsule through pores in the skin and this capsule hardens to make a water-proof cyst which remains during the dry season and is dissolved again upon the addition of water. Janson ('93) thinks it is not unreasonable to suppose that a gelatinous covering is secreted. He reasons that since the pedal glands (which are undoubtedly derived from the hypodermis) are known to secrete a sticky substance and since the tube-dwelling forms secrete a slime from their skins which helps to make the tube, then the forms which survive desiccation might easily do the same. He admits, however, that he has not found the actual secreting tissues.

Jacobs ('09) found, as a result of staining reactions with methylene blue, that the integument in the trunk is undoubtedly different in its chemical nature from that of the head and foot. He says: "The fact that that part of the cuticle which alone is exposed at the time of drying should be of a different nature from the remainder is probably significant." Jacobs is convinced that no water-proof cyst is secreted and he suggests that the thickened integument of the trunk region may be a means of preventing a too rapid evaporation as dryness comes on.

While I have no cytological evidence to show that the suggestion of Jacobs is correct or incorrect, certain it is that there is no thickening by a secretion or otherwise of the integument in the dried condition. Fig. 16 shows that instead of the integument being thicker it is actually much thinner than it was in the undried condition. The cuticle shows no great change in thickness. This would be expected in a non-protoplasmic structure. The hypodermis, however, which in the undried animal is from two to four times as thick as the cuticle, has shrunk until it is scarcely thicker than the cuticle itself. The nuclei do not shrink perceptibly but cause swellings on the hypodermis at the points where they occur. I have examined sections of hundreds of specimens dried in various ways and know that this is the characteristic condition of the integument of the dried rotifer. I believe that these observations should effectively dispose of the arguments of those who maintain that the drying animal secretes a water-proof cyst for protection during the dry periods.

In the vitellaria the drying process affects the cytoplasmic portion in less striking fashion than it does the nuclei. The granular material of the cytoplasm appears almost the same as in the undried organs. The spaces between granules are less noticeable. These spaces are probably filled with cell-sap in the active animal and it is to be expected that with loss of water they will largely disappear, permitting the more solid granules to pack closely together. The membrane surrounding the vitellarium and ovary shows no marked change. It is of practically the same thickness and consistency in the dried as in the undried animal.

In the nuclei of the vitellarium noticeable changes have occurred. As was stated before, a section of the nucleus in the vitellarium of an undried animal shows a central, densely staining karyosome surrounded by a clear space, and around the clear space a definite, but not usually thick, nuclear membrane. The nucleus in the dried vitellarium loses, for the most part, its affinity for stains. The karyosome may entirely disappear but usually there are remnants of it distributed here and there through the nuclear space. Taking the place of the karyosome is a more or less regular reticulum which reaches to the nuclear wall. The latter has become thickened during the process of drying; whereas in the nucleus in the undried condition the densely staining material was aggregated in the center and the clear area around the periphery, in the nucleus in the dried condition these relationships are just reversed. What remains of the chromatic material has collected close to the nuclear wall and the central area shows a more or less clear condition. This change in the position of the chromatic material in the nucleus is not the only one incident to the drying process in the vitellarium or in the other organs as a comparison of the figures will show, but it is by all means more frequent than the other changes.

The condition of the ovary in the dried animal is best shown in Fig. 21, Plate V. It will be remembered that the cytoplasm of the undried ovary usually shows as a glassy, homogeneous ground substance and that the nuclei appear as dense, spherical bodies closely packed in this syncytial ovarian cytoplasm. In the dried ovary the cytoplasm becomes more deeply staining and assumes an appearance not unlike that of the vitellarium. The nuclei become less dense and the chromatic material collects in a peripheral ring as was described for the vitellarium nucleus.

In Fig. 21, Plate V., a trough-like depression is seen at the margin of the vitellarium which lies nearest the integument. This is caused by one of the band-like, circular muscles, which, drying in the contracted condition, caused a deformation in the vitellarium tissue which shows thus in longitudinal section.

At this point it might be well to comment upon the fact that many of the muscles in the rotifer body actually dry while contracted. It would seem that when the normal moisture content was removed from a muscle that it would have a tendency to relax and in this way cause the animal to become more loosely folded during the later stages of drying. That this is not true is evident from the figures and descriptions given. The rotifer remains tightly contracted during the most complete conditions of desiccation.

This condition may be due to two factors. In the first place the integument is the first structure to dry. The moisture evaporates from the surface more quickly than from the internal structures. This integument is chitinous in its outer layer but is of such consistency that while moist it is very pliable. When the moisture is removed, however, the cuticle becomes more rigid. In this respect it might be likened to gelatin and such a resemblance is indeed noticeable. Now with the withdrawal of moisture the cuticle could form a rigid capsule which would retain the original shape of the folded animal even if the muscles did relax.

On the other hand it would seem that if the maintenance of the shape of the folded animal was due to the action of the cuticle the muscles would be somewhat flattened in cross-section as a result of the mutual pressure of internal organs and integument. This last condition, however, is not true, for the muscles in the dried animal still show in cross-section something of their oval outline. It seems probable that there is a limit to the amount of drying which a muscle may undergo and at the same time continue to exert a contractile tension. But the amount of drying necessary to cause a muscle to reach this limit would at the same time withdraw enough water from the cuticle to cause it to become rigid. I think, therefore, that we can account for the lack of relaxation at the time of drying by assuming that both of the factors mentioned above acting in succession produce the given result.

The appearance of the mastax and its surrounding salivary glands in the dried animal is shown in the central part of Fig. 16, but perhaps better in Fig. 25. Upon comparison with Fig. 24, which is a section of the undried mastax and glands, it will be seen that the entire structure has collapsed and decreased perceptibly in size. The gland tissue is more dense as to its cytoplasmic content while the nuclei, although just as prominent as before, show the same rearrangement of chromatic material as was described for the nuclei of other tissues in an earlier paragraph. The details of this chromatic movement given for the nuclei of the vitellarium and ovary will apply here and need not be repeated. The same is true of the foot-gland cells although I have observed certain cases where the nuclei of the latter do not assume the usual ring form. This condition is shown in Fig. 19.

The stomach of the dried animal shows, perhaps, the most remarkable changes. In Fig. 16, lying below and at the side of the mastax, is figured the stomach in its dried condition. The cytoplasmic syncytium still preserves its alveolar appearance. The lumen is shown winding about through the syncytium, while nuclei, made up of the chromatic ring, with central clear space, are scattered irregularly about. The densely staining granules of food material, so prominent in the sections of the undried stomach, are not present here. I have examined large numbers of sections of dried animals, and while the food granules are not always entirely absent, as figured here, yet they are absent in many cases, and certainly in all cases they are much less numerous than in sections of the stomach of undried animals. This point is of importance as bearing upon the question of metabolism in the dried state. As was previously stated, the identity of these granules as particles of food material can hardly be questioned. Zelinka ('88) has called attention to the fact that they do not appear in sections of the stomach of the newly hatched rotifers. Beauchamp ('09) has shown that in sections of the stomach of *Hydatina senta* which had been feeding regularly these granules were present in great numbers, but in sections of the stomach of starved individuals they do not appear. Many authors have commented upon the fact that among those rotifers which survive desiccation the stomach tissue in gross appearance is generally lighter in color after recovery than it was before drying commenced. This observation I have repeated many times and find it to be correct. I have not, however, been able to find in the literature figures of actual sections of the stomach of rotifers recovering from desiccation. Fig. 16, which is but one of many that I might show, by the absence of all food particles demonstrates that metabolic or at least katabolic activities must go on in the stomach tissue of the dried animal.

Of great importance in connection with the question of the nuclear changes is the determination of the exact time at which the chromatic rearrangement takes place. It is a reasonable supposition that it could not easily be brought about while the moisture is entirely absent from the tissues. Since the protoplasm is always more or less fluid in nature in its normal condition, the changes described would certainly have to take place very slowly if they occurred in the dried state. It would seem, upon theoretical grounds, that the more favorable time for the changes to take place would be during the short period just before actual drying occurs. At this time the tissues contain the usual amount of moisture but the approach of the dry condition is, at the same time, apparent to the animals. That there can be no doubt about the preparation of the animals for the dry state is evidenced by their behavior at the onset of desiccation, before described.

Sections of animals killed at the moment when the last visible traces of moisture were disappearing, confirm the supposition outlined above. The nuclei of the cells of the different tissues plainly show the remarkable changes which are going on and which I have described in an early part of this section. While in many cases the rearrangement of chromatin is not so complete as in some of the sections of dried animals here shown, yet it was definite and uniform in the different tissues and indicates beyond question that the transitional period for chromatic rearrangement occurs just before the last traces of moisture are removed.

The movement of chromatin just before drying is interesting for the light it throws upon the question of the lability of the nuclear material. Chromatin undergoes changes of form, position and chemical structure at the time of mitotic activity but there are few cases recorded where such changes occur during the resting condition. In the present instance, however, we have a marvelous rearrangement of the nuclear materials which occurs as a vegetative rather than a reproductive process, the essential steps of which may take place in a few minutes and the new internal conditions so established enabling the animal to resist an unfavorable environment for years.

In this connection also lies a clue to the solution of the question of mortality among rotifers which have been dried. Since under the most favorable conditions of drying some few animals never survive, it seems that there should be some definite cause to account for the fact. Comment has been made concerning the tearing of the organs of a rotifer which might result from too rapid drying. In cases where this last factor cannot enter there are still some fatalities and these cases seem to admit of explanation upon the basis of lack of time or vitality to bring about the internal rearrangement of cell elements necessary in resisting the dried condition.

A further confirmation of the fact that the changes in cell-structure take place early in the desiccation process is found by comparing sections of animals which were dried and kept in the open air with those of animals kept days or weeks in an evacuated desiccator. The essential features of cell structure and arrangement are the same in both cases. This certainly seems to indicate that the extent of the adaptive, structural response to the new condition is not directly proportional to the intensity of the stimulus.

The nuclear membrane undergoes no disintegration or other visible change in structure during the drying process. It might be supposed, from a consideration of the movements of the chromatic material into the cytoplasm, that the membrane would break up during the process. Such is not the case. Not only does it remain intact during the drying but it actually thickens. This thickening is of course due to the migration of chromatic material which normally occupies the middle of the nucleus. The chromatin proper cannot be distinguished from the nuclear membrane, however, since both react alike to stains. The nuclear membrane, then, is a persistent cell organ and does not atrophy as a result of the abnormal conditions.

It is interesting to speculate whether the material which the nucleus imbibes upon recovery from desiccation is the same as that which passed through the nuclear membrane at the time of drying. I have not been able to make any of the finer microchemical tests upon this substance but it would seem that the material which passes from nucleus to cytoplasm takes part in the oxidations discussed in a later section. The chromatin remaining in the nucleus probably manufactures new nuclear material from elements imbibed from the cytoplasm upon addition of water.

The conditions described in the last paragraph show that there are probably two kinds of chromatic material present in the nucleus, one of which is able to pass out into the cytoplasm and one which is not able to do so. Judging from the varying amounts of chromatic material which are present in different cells of the same kind when subjected to similar conditions it seems that the non-diffusable chromatin may, when conditions warrant, change chemically so as to become able to pass through the nuclear membrane. When moisture is again available the reverse process could take place and new chromatic material be built up from cytoplasmic substance. This certainly would fit in with the changes which one is able to observe in the different stages under the microscope.

The changes in the chromatin just mentioned are not unlike those which Heidenhain ('94) has described. His oxychromatin and basichromatin could be demonstrated by staining reactions. In the rotifer cells, however, the staining is not nearly so precise and delicate in the dried tissues so that it would be difficult to say whether or not the chromatin exists in the condition just mentioned. Certain it is, however, that there is a change in the chemical nature of some of the chromatin at the time of drying and a return to normal conditions when moisture is added·

D. *Animals Recovering from Desiccation.*—The condition of the organs in rotifers recovering from desiccation is shown in Fig. 15, Plate IV. This section was made from an animal which after drying thoroughly had been put into water and then killed four hours subsequent to the addition of water. This perhaps represents a case where recovery was slower than usual, but the condition of the tissues shows that it certainly would have recovered completely.

In this same section the vitellaria are seen to be resuming their normal condition. The cytoplasm is not different from that of the normal tissue. One of the nuclei has completely recovered while the other two which appear in the section are rapidly assuming typical structure. One of the first changes noticeable in the nuclei of cells of dried animals subjected to moisture is the increase in thickness in the chromatic ring in the nucleus and the greater affinity for stains exhibited by it. This is well shown in these vitellarium nuclei. The two conditions of the nuclei shown are therefore two stages in the process of recovery. Fig. 23 represents a longitudinal section through the ovary-vitellarium of an animal which had been kept in an evacuated desiccator for fifteen days, then placed in water and killed one hour and fifteen minutes after the addition of water. The recovery of this animal was more rapid than was that of the one represented in Fig. 15 for it had already partially unfolded at the time it was killed. The nuclei here show several chromatic patterns not figured in the other sections but they are all stages incidental to the resumption of the typical nuclear form.

Fig. 22 shows a transverse section of the vitellarium of an animal dried at room temperature, then placed in an evacuated desiccator for six days, then in water for one hour and finally killed and sectioned. The nuclei here show almost complete recovery, while the cytoplasmic part of the structure has not regained the characteristic regular pattern.

Another condition found frequently in the cytoplasmic portion of the vitellaria of rotifers recovering from desiccation is that represented in Fig. 20. The vitellarium nuclei are found in various stages of recovery, while entirely outside of the nuclear membrane and indistinguishable in appearance from ovarian nuclei are round chromatic particles. This condition is important for I believe it shows what we have not been able to demonstrate visually before, namely, that the withdrawal of water causes the chromatin in the vitellarium nucleus to diffuse into the cytoplasm, but the very withdrawal of water initiates a chemical change which causes the chromatin to lose its staining power. The addition of water causes this same chromatic material, scattered about through the cytoplasm to resume its normal staining reaction and in that way its actual presence is for the first time visually demonstrated. Gradually this extranuclear chromatin in the vitellarium disappears and it is probable that its disappearance is due to some chemical change which again causes it to assume the appearance or become a part of the regular cytoplasmic structure.

In Fig. 23 the cytoplasm of the ovary shows that it has regained its clear, homogeneous appearance. The number of ovarian nuclei is also greater than in sections of that organ which have not been recently dried. I have found this condition repeatedly in sections of animals recovering from drying. This is clearly one of the steps incident to increased reproductive activity. There are several factors involved in this process. It will be recalled that in drying the cytoplasm of the ovary becomes dense and takes chromatid stains more readily than do sections of the ovary of animals in the free-swimming condition. Since, as we have seen, chromatic material passes from the nuclei of the vitellarium and of other tissues into the cytoplasm of the same structures it is not improbable that a similar movement would occur in the ovary. In the latter case, however, the elements are so small that one detects minute changes with difficulty even under the highest powers of the microscope.

This condition in the ovary of the recovering rotifer seems to indicate the time and manner of the increase of the ovarian nuclei.

It is well known that periods of great reproductive activity always follow periods of desiccation. Reproductive activity under normal conditions in most animals would involve an increase in the number of the sex cells. Now in the rotifer this increase might be looked for just before drying, for, if the cells of the other tissues prepare themselves for the new conditions by rearranging their elements it is not inconceivable that the sexual elements might likewise prepare for a season of unusual activity. Such, however, is not the case. By counting the nuclei in several hundreds of sections of ovaries in normal, dried, and recovering animals, I find that the average number in the first two conditions is about the same. Likewise in sections of ovaries of animals to which water had just been added there is no noticeable increase. But in sections of animals killed several hours after the addition of water one is struck by the increase in the number of the ovarian nuclei.

It would be desirable to make a definite statement as to the exact time which must elapse before the multiplication takes place but this is impossible since the time varies among different individuals. In animals dried several weeks the increase does not become apparent in any case before one and one half to two hours has elapsed subsequent to adding water to the dried animals. This time may be too short for animals whose processes go on at a much slower rate.

In connection with the observation regarding the position of chromatic bodies in the cytoplasm of the vitellarium it is interesting to note that Janson ('93) comments upon, but gives no figures of similar cases. He says: "Only twice in *P. roseola* and *Callidina* I saw the nuclei of the ovary surrounding the large nuclei of the vitellarium but I could not distinguish whether the peculiarity was connected with egg formation or was due to pressure." In this connection I would call attention to the position of three small nuclei in the section of the vitellarium of the rotifer recovering from desiccation shown in Fig. 15. This is one of the many cases I have observed where small numbers of these nuclei are found in the vitellarium. I am certain from observations on hundreds of similar sections that this condition is due to normal causes and cannot be laid to accidental pressure as Janson suggests. I have not been able to follow all the stages in the early development of the egg, but I believe that this condition is a stage in the development of the egg and is not to be confused with those cases where chromatin arising from the nucleus of the vitellarium is found in the cytoplasm of that organ.

The condition of the different gland cells in animals recovering from desiccation is shown in Figs. 15, 18*b* and 26. Fig. 18*b* is a section of foot gland cells of a rotifer which was kept for fourteen days in an evacuated desiccator and then placed in water one and one fourth hours previous to killing. The nuclei are seen to be rapidly assuming the typical condition which is represented in Fig. 18*a*. The cytoplasmic changes in these glands are perhaps less marked than in any of the other tissues considered. This may be due in part to the nature of their contents, for being impregnated with their gelatinous secretion they would show fewer changes than would other cells whose plasma is less viscous.

The salivary glands in animals recovering from desiccation show the presence of recently acquired water by the vacuoles scattered at regular intervals throughout their cytoplasm. As recovery becomes complete the cytoplasm assumes the condition shown in Fig. 24 and a typical half-recovered condition is well shown in Fig. 26. In this figure it will be noticed that the karyosomes in the nuclei are of smaller size than in the normal tissue. They have resumed the normal condition, however, in all respects except size.

In the stomach tissue of the rotifer recovering from desiccation the absence of the food granules is again noticed. The objection might have been offered in the case of the dried stomach that the failure of the food granules to become apparent was due not to their actual absence but to a chemical change incidental to the drying process which made them entirely lose their affinity for stains. If this were the case it seems that upon the addition of water the granules would again resume their normal staining reaction. That this possibility is not realized in fact is apparent from an examination of the stomach tissue shown in Fig. 15. The nuclei have resumed their normal appearance. The cytoplasmic syncytium of the stomach tube compares favorably with that in the normal animal, while scattered about through it are vacuoles which probably mark the previous positions of the food particles which have disappeared. In any event, the food particles have been used up and the stomach tissue has the same appearance as is found in cases where the animal is not dried, but starved.

The trochal cilia in animals recovering from desiccation under the highest magnification cannot be seen to be different from those in the normal or even in the dried animal. Absolutely no change is observable.

Upon the addition of water the hypodermal layer of the integument rapidly swells and assumes its normal thickness. It might be mentioned here again that if there were any sign of a protecting gelatinous capsule it should be observed at this point when the different tissues swell and draw apart. But here, as before, there is absolutely no sign of such a gelatinous envelope.

The cells of the peripheral layer of the brain of rotifers recovering from desiccation show the characteristic chromatic ring in their nuclei (Fig. 29, Pl. V.). This, at first sight, is remarkable because the nuclei of similar cells in the dried animals do not particularly show such a disposition of the chromatic material. However, the cells in the peripheral layer of the brain are among the smallest in the rotifer body and their nuclei are correspondingly minute. The small amount of chromatin in each nucleus could migrate toward the nuclear membrane without being especially apparent. But, as was pointed out in an earlier paragraph, the addition of water causes a swelling together with an increase in staining capacity in the nuclear material with the result that the position of the chromatin is most plainly seen not in the dry period but just subsequent to that and before a sufficient time has elapsed to permit a normal arrangement to be brought about.

The observations upon the tissues of the dried animals recorded in the preceding paragraphs show that they react similarly, with very few exceptions, to the drying stimulus. The cytoplasm becomes denser. This is of course to be expected. The nuclei, all of which have a very definite and similar chromatic pattern, undergo a chromatic rearrangement which is striking and uniform. That this chromatic movement is the visible expression of an adaptation to a new sort of environment can hardly be doubted. The resumption of normal conditions in nucleus and cytoplasm is likewise uniform in the elements of the different tissues. If any doubt existed as to the nature of the adaptive response in the desiccated condition that uncertainty would be removed upon observing the return to normal conditions as shown in sections of recovering rotifers. The significance of the chromatic movement and its interaction with the cytoplasm in the different tissues will be discussed fully in a later section.

IV. Review and Discussion.

The literature relating to the cytology of desiccation is, in the case of animals, very sparse, and, relating to rotifers, almost nothing. While rotifers have been studied since the invention of the microscope, the chief interest has been along anatomical lines. Along with the anatomical studies, the Bdelloideæ have received consideration in discussions as to whether they actually dry up and recover from the desiccation, but apparently no one thought to examine the cytological changes underlying the process.

Pfeffer, in his "Physiology of Plants," makes numerous observations upon the subject of desiccation among members of the plant kingdom. His point of view is, of course, physiological rather than cytological.

Pfeffer suggests that where death occurs as a result of drying it may be due in part to the removal of the last traces of absorbed or combined water. He says: "Since full turgor is restored in mosses and other plants immediately after moistening them, it is evident that the osmotic materials remain as solids in the central vacuole. Evidently, therefore, the protoplasm is not injured by the concentrated cell sap. If, however, the latter is responsible for the death of certain plants on drying, we have revealed to us in such cases the immediate cause of the fatal action of desiccation. It is, however, hardly likely that the death of plants killed by the removal of more or less of the imbibed water, as well as that of those only killed by the removal of the last traces of absorbed or even combined water, is alike produced in the same way."

I believe that the work of McDougal, Long and Brown, mentioned in the next paragraph shows that plants may have their cell sap concentrated in the fashion described by Pfeffer and still recover from the process. My own observations upon *Philodina* lead me to think that death at the time of drying cannot be attributed to such a cause but rather to a lack of a complete adaptive rearrangement of cytoplasmic and nuclear material.

Among the botanists the subject of desiccation is now being studied by McDougal and his associates at the Desert Laboratory at Tucson, Ariz. A recent paper from that laboratory deals with cytological phenomena in the desiccation of *Echinocactus*. J. G. Brown, who did the cytological part of the work, examined four kinds of cells in the tissues of *Echinocactus wislizeni*, viz., integument, palisade, outer cortex and deep cortex. Starting with a description of these cells in sections of a plant grown under normal conditions he compares similar cells from sections of (1) a plant which had been desiccated six years; (2) one which had been desiccated ten months; and (3) one which had been desiccated forty-two months and then allowed to grow under normal conditions for twenty-two months.

In the palisade cells the results of desiccation were most apparent. These cells are rectangular in section, having a peripheral layer of protoplasm embedded in which are the nucleus and plastids. A corresponding cell from a plant desiccated six years showed the cytoplasm gathered in one corner of the cell. The nucleus, which normally is of lenticular form and contains vacuoles, was still embedded in the cytoplasmic mass. It had decreased to one fourth or less of the size of the normal nucleus. The vacuoles had disappeared and the chromatic elements had for the most part become aggregated in a ring in the region of the nuclear wall.

In the palisade cells of a cactus which had been desiccated only ten months the cytoplasm did not deviate from the normal condition as in the first case mentioned, at least in so far as its position was concerned. The nuclei decreased almost as much in size and assumed practically the same form as those in the palisade cells desiccated six years. In the specimen which was desiccated forty-two months and then returned to normal conditions for twenty-two months, the cytoplasm was found to be normal as to quantity and position, while the nuclei, although increased slightly in diameter, still retained the ring form characteristic of the dried condition.

In the other tissues the results were quite similar to those recorded for the palisade cells. In the integument the cuticle thickened slightly in the specimen desiccated six years. The outer epidermal cells were thinner in the plant dried six years than in that dried only ten months, while in the one desiccated forty-two months and returned to normal surroundings for twenty-two months the epidermal walls were of about the same thickness as in the normal tissue.

The nuclei of the epidermal cells decreased to about one half normal size in *Echinocactus* No. 7. The characteristic peripheral chromatic ring appeared. In specimen No. 6 which after desiccation was returned to a normal environment, the epidermal nuclei résumed their normal condition.

In the cells of the outer cortex below the palisade layer, the cell walls increased perceptibly in thickness as desiccation proceeded and they recovered somewhat their normal thickness as moisture was again admitted. The nuclei shrunk to about half size and assumed the ring form. In the deeper cortex practically the same things happened as were just described for the outer cortex. The cytoplasm decreased almost to the point of disappearance in the cactus dried six years and gradually increased in volume in the plants subjected to less severe conditions. The nuclei decreased to about half size. The cell-walls thickened enormously in the extreme cases of drying and in many cases never recovered normal thickness.

The results of McDougal and Brown are most interesting when compared with my own. The observations upon the cytoplasmic and nuclear changes in the dried tissues show that they are similar in many respects with those in corn embryos and rotifer tissues described in my earlier paper.

The length of time during which an *Echinocactus* may survive in the open at the expense of its surplus food material and water was found to be no more than two years while similar plants in diffuse light were sound after six years of starvation. The cactus, then, which normally lives in an environment containing little moisture is less able to adapt itself to extreme dry conditions than is the rotifer which normally swims about in water. The extreme length of time during which a *Philodina* may remain dry is not known but well authenticated records show that they have been known to withstand a period of dryness for as much as twenty-seven years. To date no experimental data are recorded to show the effects of varying intensities of light upon the time during which rotifers may live in a desiccated condition. This point I hope to treat in a future publication.

Another result of interest in the cactus experiments is that in extended desiccation and starvation the plasmatic colloids are eventually broken down by katabolic action. This katabolic activity includes hydrolysis of the cell-walls of the cortex. Now in *Philodina* katabolic activity undoubtedly takes place during the dry periods. This was most clearly seen in the tissue of the stomach-intestine. But while the reserve food granules disappear, no change is observable in the walls of any cell. It might be said from the nature of the two cases that the reaction in the cactus is irreversible while in the rotifer it is reversible, for the cells of the cactus perhaps never assume a perfectly normal condition after the drying process while a sufficient food supply is all that is necessary to make the rotifer quickly resume its normal structure.

McDougal found that in the cactus the loss in weight in full illumination may not greatly exceed 50 per cent. of the water present without producing death by desiccation. Now under normal conditions about 95 per cent. of the weight of the cactus is made up of water. This would mean that a decrease of approximately one half in the weight of the plant as a result of drying, is usually fatal. In *Philodina* the minuteness of the animal, together with the fact that it normally lives in water, makes it almost impossible to determine the changes in weight during the drying period. It is easy, however, to follow the changes in volume which occur during drying and anyone can see if he will dry a *Philodina* upon a slide under a microscope that the animal decreases to from one third to one fourth of its original size. Since most of the weight of the rotifer body, certainly more than 95 per cent., is made up of water it will be seen at once that the percentage of substance lost by the rotifer during desiccation is much higher than that observed in the cactus.

The desiccation process in the cactus caused few changes in the thickness of cell-walls. In the plant treated for seventy-three months the cuticle was slightly thicker than normal while the outer walls of the epidermal cells were thinner. This result was obtained in the most severe test of the series. In my experiments on *Philodina* the most severe drying caused absolutely no thickening of the cell walls which bordered the surface, but on the contrary by a loss of water from the hypodermal layer an actual decrease in the thickness of the integument was observed in all cases.

Perhaps the most pronounced cytological effects of desiccation are those recorded for the cortex cells of *Echinocactus*. Here there was an entire disappearance of the protoplasts and a hydrolysis of the cell walls. There is nothing in my experiments upon *Philodina* which parallels this. The changes described in the stomach tissue are similar but in no case have I ever observed the disappearance of cell-walls or the formation of spaces as a result of the disintegration of cell-elements.

That the different changes during the desiccation process and subsequent recovery in *Echinocactus* are very slow is well shown by McDougal and Brown when they state that a plant which had been desiccated for forty-two months and was then placed under normal conditions in the soil for twenty-two months did not entirely regain the normal condition. It would be interesting to know if the plant would have recovered if given more time for the process. In *Philodina* the process of recovery begins upon the addition of water and is complete in a short time, never longer than a few hours.

For an analysis of the meaning of these changes I should say that the cactus is unfavorable for rapid results. The great length of time necessary for changes to take place and the slowness with which normal conditions are resumed makes experimental work with a large number of specimens difficult. This probably accounts in part for the fact that McDougal and Brown make no attempt to suggest the significance of the cytological changes which they describe.

Metabolic Water and Its Relation to Desiccation.

For a complete discussion of the subject of metabolic water and its functions in an organism I would refer the reader to the excellent paper of Babcock ('12). For the present purpose it will be sufficient to outline the main points of the subject and relate them to the material under discussion.

Desiccation is the operation of drying or removing water from a substance. When the last trace of water is removed the desiccation is said to be absolute. Absolute desiccation of chemical crystals is brought about by heating the substance to dryness or when the material involved is readily decomposed by heat the operation is accomplished by the use of a desiccator. A condition of absolute desiccation can be maintained so long as the heat is applied in the one case or so long as the substance is kept within the drying chamber in the other. When the desiccating influence is removed moisture is absorbed.

Inorganic crystals can, as a rule, be deprived of their moisture only by a temperature of 100 degrees centigrade or higher. Not only are the crystals as such made dry but the water of crystallization, by virtue of which the crystals exist, is driven off and the substance assumes an amorphous condition.

Living organisms contain water combined with them in a fashion which for present purposes may be compared to water of crystallization. In order to remove all water by means of heat it is necessary to raise the temperature of the organic material in question until actual charring takes place. It is evident, therefore, that the absolute desiccation of living substance cannot be accomplished by means of heat without destroying life. On the other hand, the second method of desiccation mentioned above will not remove the last trace of chemically combined water from living tissue. Since an absolute desiccation of living things without the destruction of life is impossible by the second method it seems certain that no rotifer or other living thing has ever lived after an absolute desiccation.

The recorded observations upon the desiccation of living things, whether animal or plant, have to do with a relative rather than with an absolute desiccation. If the water content is lowered much below the normal percentage the organism may be said to have undergone an actual or even a complete desiccation but it will be understood that in all cases this does not imply that the condition of dryness is absolute.

Water may be acquired by an organism in three ways: (1) It may be imbibed directly; (2) it may be taken in with solid food, and (3) it may be formed within the organism by metabolic changes in the organic constituents of the food and tissues induced by respiration and other vital processes.

Imbibed water probably makes up the greater part of the cell-sap. Its method of combination with the living substance may be, as Nägeli contended, not chemical but a mere physical attraction due to minute molecular aggregations designated as micellæ, between which the water enters by capillary attraction and forcing the micellæ apart increases the volume of the tissues. On the other hand, as Babcock points out, all of the phenomena of imbibition point directly to a molecular combination between the substance composing an organized body and water. The combination is in most cases feeble, since it is broken up by a relatively low temperature without changing the molecular structure of either the solid tissue or the water. It is however analogous to the behavior of many substances, both organic and inorganic, which crystallize with water of crystallization.

The water taken in with solid food probably has in part the same fate as imbibed water although its history is slightly different. In *Philodina* the distinction between these two sources of water is not marked and the same can be said of any animal having a similar habitat and feeding habits.

The production of metabolic water, in certain stages of the life-histories of both plants and animals is sufficient for all purposes for considerable periods of time. In the resting periods of deciduous plants, in bulbs, in tubers and especially in seeds and spores, ample water is provided for all vital processes by the slow oxidation that takes place as a result of direct respiration. Hibernating animals receive no water from external sources for several months, although water is being constantly lost by respiration and excretion. Many varieties of insects such as clothes moths, grain weevils, dry-wood borers, etc., are able to subsist during all stages of development upon air-dried food materials containing less than 10 per cent. of water; in these cases nearly all the water required is metabolic.

Metabolic water may be formed as a result of two kinds of respiration. In *direct respiration* the organic matter comprising the food and tissues of an organism is oxidized by means of free oxygen derived from the air during respiration. Many organisms when deprived of free oxygen are capable of maintaining for a short time certain of the respiratory functions, and deriving energy from food material and from tissues by breaking up the molecular structure into new forms of a lower order. This is known as *intramolecular respiration.*

When a *Philodina* dries it loses all its free water. The spaces between the different organs are filled during the free-swimming existence of the animal, with fluid. This fluid between organs is the first to disappear. The loss of uncombined water is responsible in large measure for the decrease in size of the animal during the drying process. By examining Figs. 1 and 2 it will be seen that in their normal condition the organs are not closely packed together. It is these spaces between organs which allow the animal to decrease so appreciably in size and in such a decrease the fluid content of the spaces is lost.

In addition to the loss of free or uncombined water there is a corresponding though not extensive loss of combined or chemically bound water. This is the imbibed water mentioned in a previous paragraph. Just as water is distributed in the interstices of a sponge, so water is held in every cell of the rotifer body. It is hardly necessary to emphasize again in this connection that such water is probably not held by capillary attraction as in a sponge but by a loose molecular union. The presence of this water in the rotifer tissues is demonstrable in a visual fashion, for in the sections of normal undried rotifers the cells all possess vacuoles between the granules or reticulum of the cytoplasm. These vacuoles or spaces are certainly filled with the more fluid cytoplasmic ingredients and their true fluid nature is demonstrated upon examination of a section of a dried animal where they have with few exceptions disappeared, or better still in an animal recovering from desiccation where the former position of granules of reserve food material is marked by vacuoles undoubtedly containing liquid. In a previous paragraph attention was called to the fact that in those cells whose cytoplasm is loosely granular in the normal condition, the granules were closely packed in similar cells of a dried animal. The change in space relation of these cytoplasmic granules is due to loss of imbibed water.

The amount of imbibed water which an organism may lose and still live varies with the kind of organism. Seeds which in a dormant condition contain from ten to not more than twenty per cent. of water may still retain their viability and germinating power if more than half of the water content is removed. This viability of corn with different degrees of moisture content is recorded by Babcock ('12) and I have repeated many of his experiments and find them to be correct.

In McDougal's cactus experiments it was found, as was before mentioned, that loss of weight in full illumination may not greatly exceed fifty per cent. of the water present without producing death by desiccation. In seeds, on the other hand, a loss of seventy-five per cent. of the water content need not necessarily be fatal and in some cases certainly the percentage loss may be higher without death resulting.

In *Philodina*, while exact quantitative measurements are exceedingly difficult, the water loss judged by decrease in size of the drying animal and by the nature of the dried tissues, must certainly be higher than that of seeds.

The Mechanism of Metabolism in the Dried Rotifer.

Admitting that the desiccation of *Philodina* is complete and admitting further that metabolism takes place though much retarded, in the dried condition, the mechanics of the metabolic activity is the next point to demand explanation.

It will be remembered that Jacobs ('09) showed by means of intra vitam staining with chemical indicators and subsequent tests with various gases, that the integument of the dried rotifer is at all times freely permeable to gases. Attention was called, in another paragraph of this paper, to the fact that many animals when deprived of their normal supply of moisture could still exist for a time by means of the direct action of oxygen from the air upon the complex materials of the tissues or upon inclusions of complex food materials within the latter. This action would of course not be sufficient to prolong life indefinitely for the time would come when all the available reserve food material would be exhausted or the accumulation of poisonous waste products might end life. That such a state of affairs might be realized in the case of a dried *Philodina* in the air is not impossible. Certain it is that this animal can live a long time; many years in fact, in a dried condition without food from external sources. Its lease of life, however, under these conditions, is not indefinite as is evidenced by the fact that under the most careful conditions of drying some of the rotifers always die. To explain these fatal cases it would seem that the store of reserve material became exhausted or the metabolic products accumulated in too great a quantity and death was the result. It seems reasonable to assume then, that under the conditions just outlined metabolic activity goes on through oxidations of complex substances within the tissues of the animal by means of oxygen obtained by direct respiration.

Those rotifers which were described as having been kept in an evacuated desiccator for varying periods and which survived the experiment could not have their metabolic activities explained upon the same basis as the ones which were dried in air. It will be recalled that mention was made of certain cases where metabolism might proceed in the absence of air by means of intra-molecular activity. In these cases the complex substances which are an integral part of, or are merely inclusions in the tissues have their molecular structure broken up into newer forms of a lower order. In this way energy is derived from food material and life may be prolonged for a greater or less period of time depending upon the amount of reserve material present.

Both types of chemical reactions outlined above are hydrolytic processes. This means then that metabolic water is being evolved in small quantities during the entire dry period.

It should be understood here that the processes just outlined are hardly possible of actual demonstration for if they are sufficient to keep such a small organism as a rotifer living for years in the dried condition they must of necessity be exceedingly slow. Observations made by Babcock ('12) show that respiration and consequent oxidations in seeds and spores are practically suspended, it being possible to detect them only by observations extending over long periods of time. If this be true of an embryonic structure with its simple organization it is true to a greater degree in an adult animal in a state of retarded activity.

Death ensues in all dormant organisms at the end of a certain time. The period of dormancy is limited. In *Philodina*, since metabolism proceeds continuously but slowly as I have shown, the death of an animal can be attributed to starvation rather than dryness. Dryness is a contributing cause of death but the animal dies as a result of lack of food rather than as a result of lack of moisture.

We may briefly summarize the previously mentioned causes of death in the dried rotifer as follows: (1) Mechanical injury due to too rapid drying, (2) starvation resulting from lack of reserve food material, (3) poisonous effect of metabolic products and (4) insufficient time before drying to effect the nuclear-cytoplasmic reorganization.

The Significance of the Nuclear-Cytoplasmic Interchange During Desiccation.

The nucleus in the cells of most of the tissues of *P. roseola* consists, as was pointed out before, of a single large karyosome surrounded by a clear area and having for its boundary a definite nuclear membrane. This karyosome is of course not a true nucleolus since it is made up of chromatic material. This type of nucleus has been given the name of "nucleoles noyau" by Carnoy and it has been described by numerous authors in the cells of several kinds of unicellular animals. Nucleoli showing apparently all grades of morphological and chemical properties between true plasmasomes and karyosomes have been described by various authors (see Montgomery, '98) so that it cannot be said that there are no intermediate conditions to be found. Montgomery says: "The existence of Carnoy's 'nucleoles mixtes' and 'nucleoles-noyaux' in cells of metazoa appears to be doubtful" yet the "pseudonucleoli" which he describes in the ova of the mollusc *Montagua pilata* have many of the properties of a chromatin nucleolus or karyosome.

The significance of the peripheral chromatic ring in the dried rotifer nucleus is a point which requires explanation and correlation with the other conditions incident to the adaptation of the rotifer to its dried condition. The chromatin ring undoubtedly represents a stage of chromatin migration. There are two possibilities regarding the extent of this migration. The chromatin may merely leave its place in the center of the nucleus and, by taking a position next to the nuclear membrane, remain within the nucleus proper during the entire dry period. On the other hand, some of the chromatic elements might be of such nature as to pass readily through the nuclear membrane while the latter might be impermeable for others. That materials may be changed chemically within the nucleus is admitted. In nuclei of cells which have just completed mitotic division the nucleus imbibes substances from the cytoplasm and changes them into nuclear material. It is also a matter of common knowledge that at the beginning of mitotic activity much material is cast out of the nucleus into the cytoplasm. However, it is not commonly contended that materials pass from the nucleus into the cytoplasm without a rupture of the nuclear membrane. That the latter condition is a possibility in the rotifer nucleus will appear shortly.

The karyosome of the rotifer nucleus being almost pure chromatin, the reason for its migration might be sought in a consideration of some of the functions of chromatin in general. R. Lillie ('02) and others have shown "that in many tissues the nucleus is the chief agency in the intracellular activation of oxygen; and further that the active or atomic oxygen is in general most abundantly freed at the surface of contact between nucleus and cytoplasm." Now the parts of the nucleus which take part in these oxidations must be the chromatin and, perhaps, the nuclear membrane. It seems reasonable to suppose that the lack of the normal amount of water in the nucleus causes the chromatin to be unable to interact with the nuclear membrane in bringing about oxidations, and the nucleus adapts itself to the new conditions by rearranging its chromatic content in the manner described.

I have implied in the preceding paragraph that the chromatic material goes no further than the nuclear membrane and that there it is deposited to make the typical ring structure. While this may be and probably is true in cases of relatively incomplete desiccation, I think there is much evidence that in many cases the chromatin either does pass through the nuclear membrane or it is so changed chemically within the membrane that its presence cannot be detected by ordinary methods. A comparison of different nuclei in the figures of sections of dried animals shows that the peripheral chromatic ring is not nearly uniform in thickness in the different nuclei. I interpret this as meaning that varying amounts of chromatic material have been able to pass to the cytoplasm,—the ability or inability to do so depending perhaps for one thing upon the permeability of the nuclear membrane.

It will be remembered in this connection that immediately upon the addition of water to the dried animals the chromatic ring thickens and regains its normal staining power. This fact also is, I think, capable of two interpretations. It may indicate that chromatic material which moved in some form into the cytoplasm during the dry period immediately begins to return to its normal position when moisture is restored or it may be that the drying process caused the chromatin to lose its staining power to such an extent that its volume only seemed to be diminished. From the appearance of the sections I do not think that there is any doubt but that the former supposition is correct. The chromatic material in some form actually does pass into the cytoplasm during the dry period.

Whatever may actually happen to the chromatin in the way of a change of position it seems certain that the purpose of all the position changes outlined are one, namely to keep the chromatin within a working radius of the material to be oxidized. The oxidations referred to in this connection are the breaking down of complex food materials in the cytoplasm with the consequent release of metabolic water during the process. The details of this process are discussed in another paragraph.

The nucleus of the gregarines is similar in many points of structure with that of the rotifer. Montgomery ('98), speaking of an unnamed gregarine from *Carinella annulata* says: "Now as the gregarine grows, at the same time both nucleus and the total mass of the nucleolar substance increase in size; but the nucleus cannot grow without the addition of a substance or substances to it which have been derived from without. Accordingly, I suppose that the substance of these granules has an extranuclear origin, a substance, *i. e.*, which having penetrated the nucleus from the cytoplasm, undergoes a chemical change in the nucleus and there becomes precipitated in the form of granules, for no such substance occurs in granular form in the cytoplasm. The growth of the nucleoli might then be explained on the assumption of the intussusception of this substance by the nucleoli." It seems probable that the rotifer nucleolus grows at the time of recovery from desiccation in the fashion outlined by Montgomery for the gregarine nucleolus. Drying then, brings about a reversal of this process and the normal condition is resumed only when moisture is restored.

In considering the movements of chromatin from the nucleus into the cytoplasm one is struck by the similarity between the phenomena here observed and those described by Woodruff and Erdmann in their paper upon periodic reorganization in *Paramœcium aurelia*.

These authors working upon Woodruff's non-conjugating line of *P. aurelia*, found that there was a periodic nuclear reorganization. "This nuclear reorganization consists of a gradual disintegration and absorption of the macronucleus in the cytoplasm. Simultaneously a multiplication of the micro-nuclei is in progress. Certain of the resulting micronuclei degenerate while the remaining one or two form the new macronuclear and micronuclear apparatus. This results in the reorganization of the cell without the fusion of two animals." They find not only that the reorganization process is coincident with the low point between rhythms but also that there is a causal relation between the reorganization process and the rhythms. Woodruff defines a rhythm as a minor periodic rise and fall in the division rate from which recovery is autonomous. Woodruff believes that the rhythmical changes which he describes are inherent in the phenomena of the cell. There are changes in *Paramœcium*, described by Calkins and others, involving a somewhat similar reorganization of the cell but whose cause may be laid in part to environmental conditions.

The life history of *Philodina* consists in periodic increases and decreases in activity as a result of environmental conditions and in many respects its structural responses are similar to those described by Woodruff and Erdmann in *Paramœcium.* The environmental stimulus acting upon the rotifer to produce the periodicity is dryness. The response to the stimulus is an interaction between nucleus and cytoplasm not unlike that taking place in *Paramœcium.* Normal conditions of organization are resumed as soon as the stimulus is removed and the noticeable result of the entire process is an increase in reproductive activity. Of course many of the physiological processes of the two animals are markedly different in many respects and would not admit of close comparison but this one particular phase in which there is such a marked agreement in the cytological phenomena accompanying a physiological state seems worthy of comment.

The Relation between Desiccation and Reproductive Activity.

It is hardly necessary to again call to mind the causal relation between desiccation and reproduction in *Philodina.* The numerous observations of many authors has established this as a fact. It is, however, profitable to speculate concerning the specific cause of the increased reproduction. It is insufficient to say that drying is the cause. It is necessary to point out if possible how desiccation causes the reproductive increase.

In an earlier paragraph I have shown that the nuclear-cytoplasmic reorganization takes place in the ovary the same as in other tissues, and that just subsequent to the addition of water an increase in the number of ovarian nuclei takes place. I have not been able to observe these elements in the act of multiplication but I am sure concerning the results of the process if not of the method.

It seems, then, that we are dealing with an adaptive, structural response of a special sort. Whereas in the other tissues the reorganization of cell elements took place to satisfy a vegetative or metabolic demand, here we have a similar reorganization, the end of which is increased reproduction, and the stimulus to which is in both cases,—desiccation.

In addition to the increase in the number of sexual elements, a stimulus is also apparently given which leads to the immediate development of some of these elements.

That egg cells should be stimulated to develop as a result of loss of water is neither a strange idea nor a new one. Loeb ('06) in his experiments upon artificial parthenogenesis found that the unfertilized eggs of *Arbacia* and *Strongylocentrotus* could be induced to develop into swimming larvæ by immersing them in hypertonic sea-water and later placing them in water of normal concentration. That the initiation of development was due to the withdrawal of water he demonstrated by further experiments in which the eggs were not put back into sea-water of normal concentration. In this case only a loss but no taking up of the water occurred, yet swimming larvæ developed.

Philodina is a parthenogenetic rotifer, and as has been shown is stimulated to reproduce by removal of moisture from its tissues and consequently from the sex cells. It would seem, therefore, that we are here dealing with a process which is natural and commonly employed by this parthenogenetic animal as a result of environmental conditions but that the same stimulus can be employed to bring about parthenogenetic development among an entirely different group of animals which reproduce normally by the sexual method. The steps in the two processes are not to be easily compared, yet the initial stimulus is similar and development is the result in both cases.

Desiccation Phenomena in Their Relation to the Subject of Adaptation.

That desiccation is but one of the unfavorable conditions to which rotifers may adapt themselves is evident from the literature. The Bdelloideæ can easily adapt themselves in other ways. Murray in his account of rotifer fauna observed during the first Shackleton south polar expedition found that *Callidina constricta* and *Adineta grandis*, both *Bdelloids*, were able to live for rather long periods in salt water although normally they are found only in fresh water. Murray in the account of his experiments says: "To test the degrees of cold which they could stand blocks of ice were cut from the lakes and exposed to the air in the coldest weather of the whole winter. By boring into the center of the blocks we found that they were as cold as the air. A temperature of − 40° F. did not kill the animals.

"Then they were alternately frozen and thawed weekly for a long period and took no harm. They were dried and frozen and thawed and moistened and still they lived. At last they were dried and the bottle containing them was immersed in boiling water, which was allowed to cool gradually and still a great many survived. Again they were put into sea water and into the brine from the bottom of Green Lake which is so salt that it only freezes at about 0° F. They were kept in these salt waters for a month, yet as soon as they were transferred to fresh water they began to crawl about as though nothing had happened.

"Such is the vitality of these little animals that they can endure being taken from ice at a minus temperature, thawed, dried and subjected to a temperature not very far short of the boiling point, all within a few hours (a range of more than 200° F.)."

It is desirable that the structural changes, if any, which accompany freezing and salinity of the surrounding medium be worked out in order to compare the resulting conditions with those in the dried animals. It is probable that when the different environmental conditions were brought about successively in varying combinations, the structural response is somewhat different in each case.

Bachmetjew ('07) showed that juices of the insect body do not completely congeal till they have been reduced to − 4.5° C., but at this temperature the insect does not yet die.

Reiff ('09) kept an adult *Actias selene* at a temperature of − 3° to − 6° C. from November 23 to January 3. Upon raising the temperature to 17° C. the insect again became active. The normal length of life of this species of *Actias* averages seven to eight days. The total number of days which the two insects experimented upon lived in an active condition was about two days less than the average.

Bachmetjew says that metabolism cannot take place in a frozen insect because it is impossible for the blood to circulate. However this may be, we have shown that metabolism takes place in the dried rotifer in the absence of a circulating fluid. Furthermore the experiments of Reiff just mentioned show that the total length of life is lessened in an insect when freezing intervenes. This strongly suggests that the life processes go on slowly here also. It seems desirable that the question of structural changes and metabolic processes during freezing among certain animals should be carefully investigated.

V. Summary.

1. The tissues and the parts of the individual cells of a desiccated *Philodina roseola* maintain their identity during the drying process.

2. No protecting membrane is secreted when drying begins or at any other time during the desiccation process. The integument of the dried rotifer is thinner than that of the undried specimen.

3. Metabolism goes on slowly in the dry condition as is evidenced by changes in the walls of the digestive tube.

4. Desiccation in *Philodina* may be complete but not absolute without fatal results.

5. The typical nucleus in all the tissues of *Philodina* consists of a single large karyosome surrounded by a clear space with a distinct nuclear membrane at the periphery of the clear space.

6. The general effect of desiccation upon the cells of the rotifer tissues is the production of a nuclear-cytoplasmic rearrangement

in which the chromatic part of the nucleus migrates to the periphery and the cytoplasm becomes more dense.

7. The movement of chromatin during desiccation phenomena takes place in order that the cell oxidations may continue during the dried condition.

8. The extent of the structural rearrangement which takes place in the rotifer cells is not directly proportional to the intensity of the stimulus.

9. In animals recovering from desiccation the elements of nucleus and cytoplasm gradually resume normal relationships.

10. The chromatic rearrangement in the nuclei of cells of drying rotifers takes place at the very beginning of the drying process.

11. The acceleration of reproductive activity just subsequent to drying is traceable to an increase in ovarian nuclei. This increase takes place while the animal is recovering.

12. The cytological changes attending a recovery from desiccation are in their nature the exact reverse of those taking place during the drying process.

13. The death of cells as a result of desiccation is probably not a result of the activity of concentrated osmotic materials upon the protoplasm as Pfeffer suggests.

14. The death of rotifers during the desiccation process may be due to one or a combination of the following causes: (1) Mechanical injury due to too rapid drying, (2) starvation resulting from a lack of reserve food material, (3) the poisonous effect of metabolic products and (4) insufficient time before drying to effect the nuclear-cytoplasmic reorganization.

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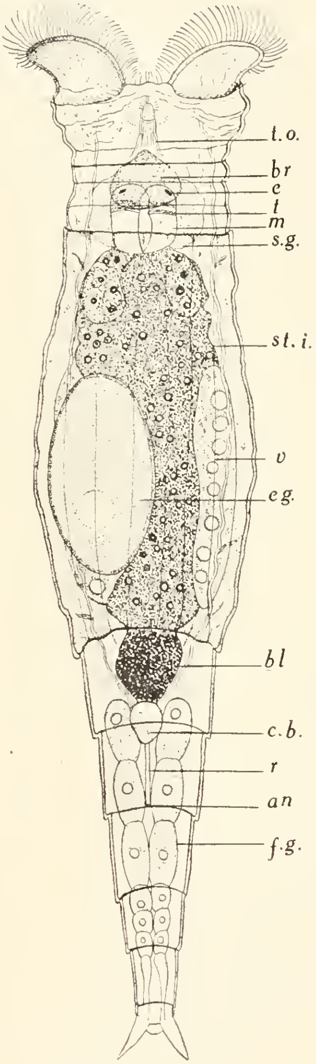
VII. EXPLANATION OF PLATES.

PLATE I.

FIG. 1. Dorsal view of a living extended *Philodina roseola*. *t.o*, tactile organ; *br*, brain; *e*, eye; *t*, tooth; *m*, mastax; *s.g*, salivary glands; *st.i*, stomach intestine; *v*, vitellarium; *eg*, egg; *bl*, "blasendarm"; *c.b*, contractile bladder; *r*, rectum; *an*, anus; *f.g*, foot-gland.

FIG. 2. Ventral view of same animal. *ph*, pharynx; *s.g*, salivary gland; *f.c*, flame cell; *e.c*, excretory canal.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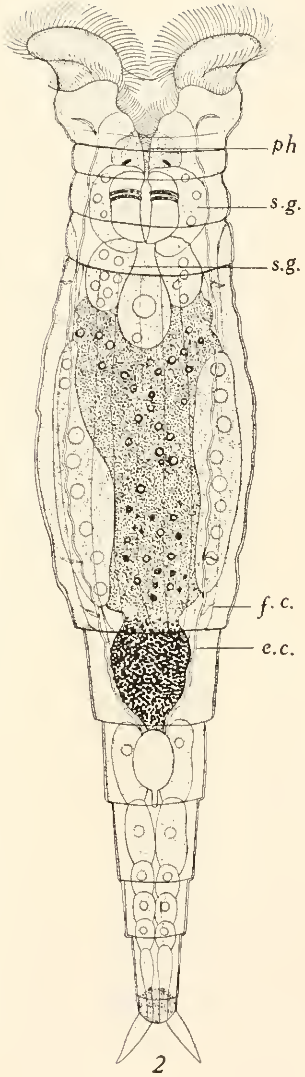
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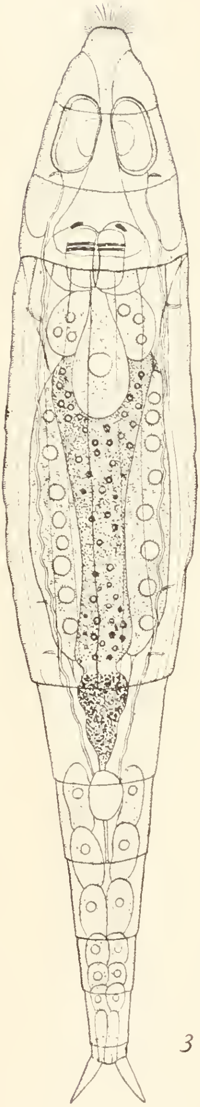
PLATE II.

FIG. 3. Ventral view of *Philodina roseola* with trochal discs retracted and proboscis extended.

FIG. 4. *Philodina roseola* in contracted condition.

FIG. 5. *P. roseola* in dried contracted condition.

FIG. 6. Side view of foot of *P. roseola* showing toes.



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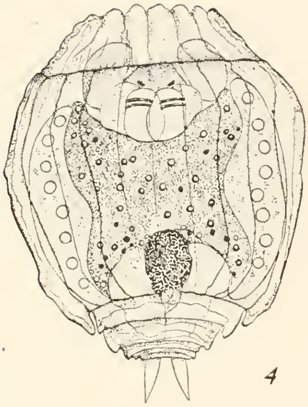

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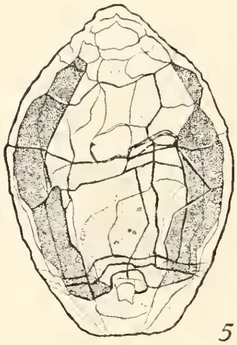

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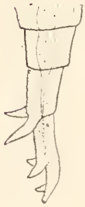

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Plate III.

Fig. 7. Section through brain and posterior part of pharynx of normal extended animal. Zeiss. Oc. 6, Obj. 2 mm.

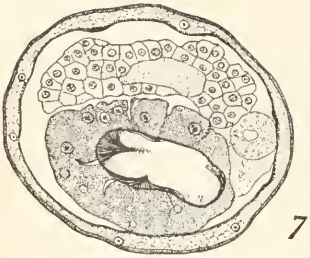
Fig. 8. Section just posterior to the mastax and through salivary glands and oesophagus. Zeiss Oc. 6, Obj. 2 mm.

Fig. 9. Section through mid-body region. Leitz Oc. 4, Obj. 2 mm.

Fig. 10. Section through mid-body region of animal containing egg. Zeiss Oc. 6, Obj. 2 mm.

Fig. 11. Section through point of juncture of stomach-intestine and "blasendarm." Zeiss Oc. 6, Obj. 2 mm.

Fig. 12. Section through contractile bladder and foot-glands. Zeiss Oc. 6, Obj. 2 mm.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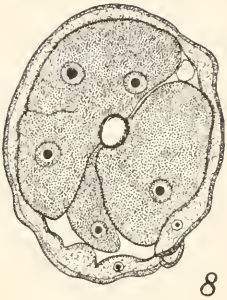
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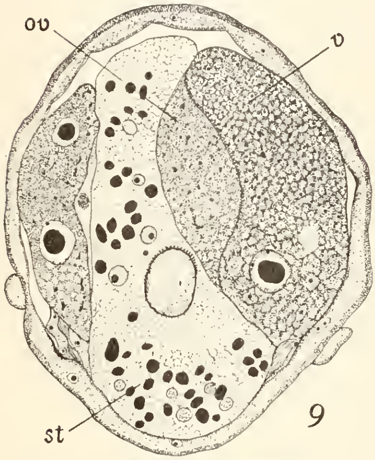

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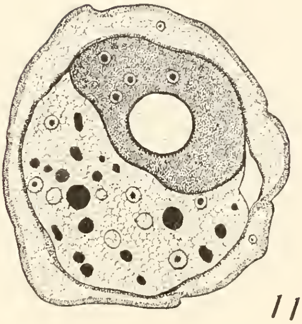
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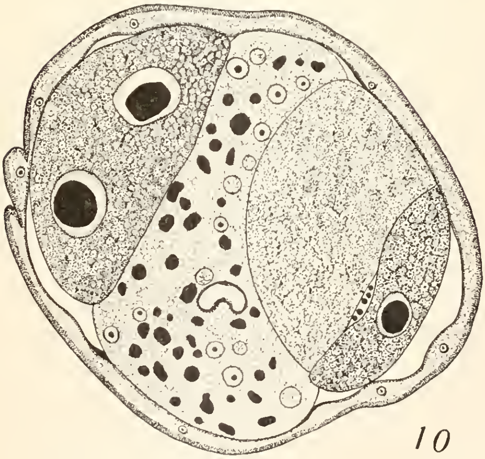
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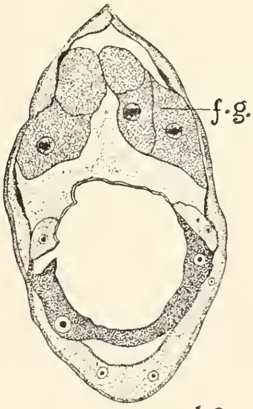

12

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PLATE IV.

FIG. 13. Frontal section through middle of contracted *P. roseola*, not dried. Zeiss Oc. 6, Obj. 2 mm.

FIG. 14. Slightly oblique section through a contracted animal, not dried. Leitz Oc. 4, Obj. 2 mm.

FIG. 15. Frontal section through middle of a rotifer recovering from desiccation. Animal was killed four hours subsequent to the addition of water. Zeiss Oc. 6, Obj. 2 mm.

FIG. 16. Frontal section through rotifer kept in an evacuated calcium chloride desiccator for eighteen days previous to fixation. Leitz Oc. 4, Obj. 2 mm.

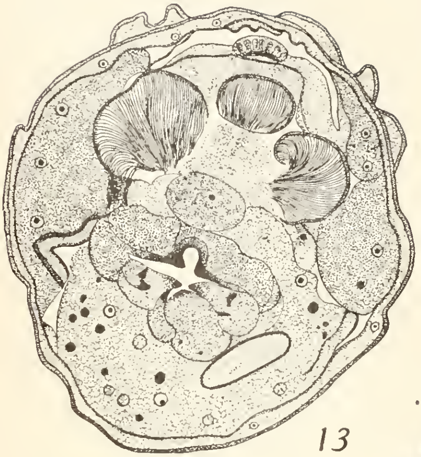
FIG. 17. Section of foot-gland cells of a rotifer which was kept in an evacuated desiccator for fourteen days previous to time of fixation. Leitz. Oc. 12, Obj. 2 mm.

FIG. 18*a*. Section of foot-gland cells of a normal active animal. Leitz Oc. 12, Obj. 2 mm.

FIG. 18*b*. Section of foot-gland cells of an animal kept for fourteen days in an evacuated desiccator and then placed in water for one and one-fourth hours previous to fixation. Leitz Oc. 12, Obj. 2 mm.

FIG. 19. Section of foot-gland cells from animal dried one week at room temperature. Zeiss Oc. 6, Obj. 2 mm.

FIG. 20. Section of vitellarium from animal dried five days at room temperature, placed in water four hours, then killed and sectioned.



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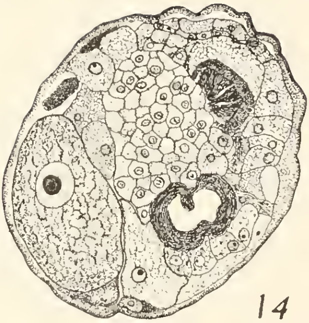

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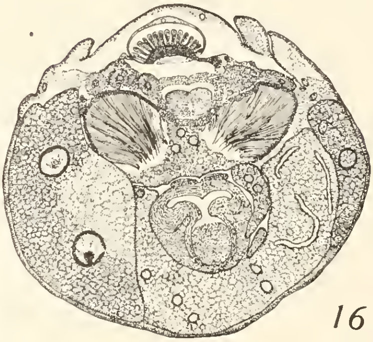

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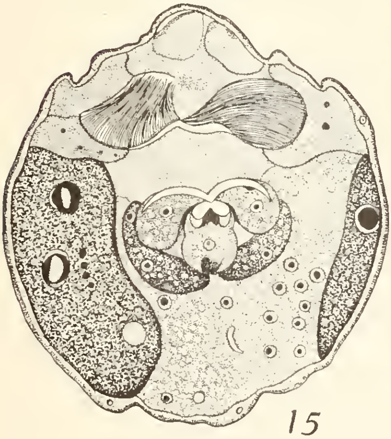

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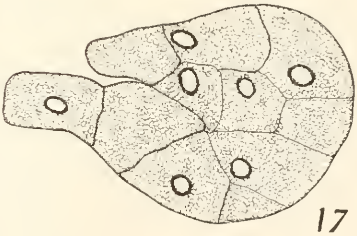

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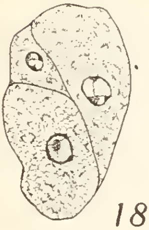

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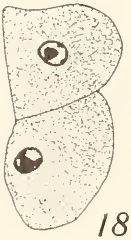

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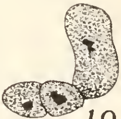

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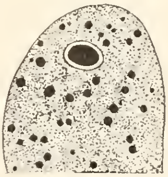


20

L. M. HICKERNELL.

PLATE V.

FIG. 21. Longitudinal section through ovary-vitellarium of a rotifer dried in an evacuated desiccator for fourteen days previous to time of fixation. Leitz Oc. 8, Obj. 2 mm.

FIG. 22. Cross-section of vitellarium of animal recovering from desiccation. Animal was kept in an evacuated desiccator for six days, then placed in water for one hour at the end of which time it was killed. Leitz Oc. 8, Obj. 2 mm.

FIG. 23. Longitudinal section of ovary-vitellarium of animal recovering from desiccation. The rotifer was kept in an evacuated desiccator for fifteen days, then placed in water for one hour and fifteen minutes at the end of which time it was fixed. Leitz Oc. 8, Obj. 2 mm.

FIG. 24. Cross-section of mastax and salivary gland of a normal undried animal. Zeiss Oc. 6, Obj. 2 mm.

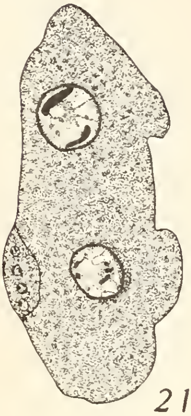
FIG. 25. Section of mastax and salivary gland of an animal dried in an oven at 40° Centigrade for four days. Zeiss Oc. 6, Obj. 2 mm.

FIG. 26. Section of salivary gland cells from an animal desiccated, kept four hours in water, and then killed. Zeiss Oc. 6, Obj. 2 mm.

FIG. 27. Cross-section of brain of *P. roseola*. Normal active animal. Zeiss Oc. 6, Obj. 2 mm.

FIG. 28. Cross-section of brain of a rotifer kept in an evacuated desiccator for fourteen days previous to time of fixation. Leitz Oc. 8, Obj. 2 mm.

FIG. 29. Cross-section of brain of *P. roseola* dried twenty-four hours, put into water for one hour and then killed. Leitz Oc. 8, Obj. 2 mm.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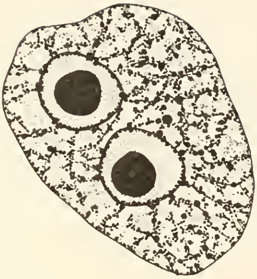
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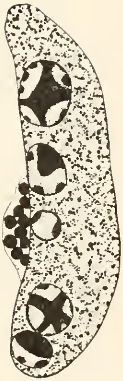
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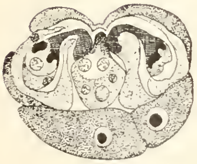
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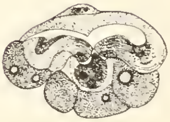
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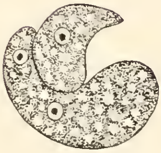
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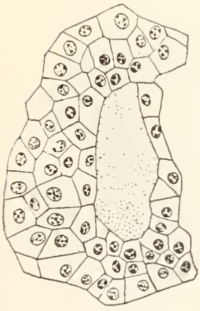
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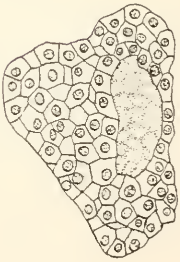
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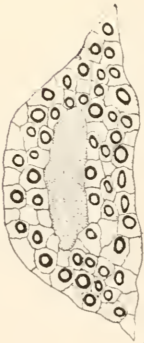
29

L. M. HICKERNELL

MITOCHONDRIA AND OTHER CYTOPLASMIC STRUCTURES IN THE SPERMATOGENESIS OF PASSALUS CORNUTUS.

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A. Introduction.

In course of my study on the spermatogenesis of *Passalus cornutus* (one of the Lacunid beetles), the mitochondria and other cytoplasmic inclusions came to my attention. Although I was primarily interested in the study of the nuclear changes with especial reference to synapsis, it seemed desirable to make a study of the cytoplasmic structures above mentioned, since they showed so clearly in my preparations. I shall therefore reserve for a later report a description of the nuclear changes involved in the course of spermatogenesis.

I am glad to acknowledge my indebtedness to Prof. E. G. Conklin, of Princeton University, under whose guidance and criticism this work has been pursued. I also wish to thank Mr. Milton P. Hunter, of Westtown, Pa., from whom I received the material used in this study.

B. Material and Technique.

There are two pairs of more or less bulb-shaped testes situated in the posterior part of the abdomen. These were dissected out in Ringer's solution and immediately fixed in one of the following fixation fluids: Hermann's, Flemming's strong solution, and Benda's modification of Flemming's fluid. Iron hæmatoxylin followed by either Lichtgrün, erythrosin or Bordeaux red was the stain most used, especially when the fixations were with Hermann's or Flemming's fluids. The Benda crystal violet-alizarin staining method was employed on the material fixed in the Benda fluid. On the whole, material fixed in strong Flemming's fluid (three or four hours) and then stained in iron hæmatoxylin followed by one of the counterstains previously mentioned, was the most satisfactory. In Hermann and Benda material there is a tendency, especially in the spermatocytes, for the mitochondria to agglutinate in dense irregular masses.

C. OBSERVATIONS.

(*a*) *Chromosomes.*

Although not primarily concerned with the chromosomes in the present report, it may be of some interest to at least mention these in a general way. I have as yet been unable to find spermatogonial plates of sufficient clearness to make accurate drawings, but all counts indicate that the chromosome number is 26, including an unequal pair. This has been confirmed by a study of the ovaries in which all dividing follicle cells show 26 chromosomes and these can be arranged in equal pairs (Fig. 3). The metaphase plate of the first spermatocyte (Figs. 12, 33) shows 13 bivalent chromosomes, one of which represents the unequal (sex) pair. The elements of the sex pair separate in the first maturation division and divide equationally in the second division.

(*b*) *Mitochondria.*

I shall not attempt to review the vast literature which has grown up bearing on the subject of the mitochondria. Duesberg ('11) has given a rather complete review of the literature, so that it will not be necessary to do so here. I shall, however, discuss the works of others insofar as these may be related to my observations on *Passalus*.

1. *Spermatogonia.*—In the primary spermatogonia, which are situated at the blind end of the testes, I have been unable to find conclusive evidence of the presence of mitochondria (Fig. 1). The cytoplasm is usually of a homogeneous, non-reticular and finely granular structure. These granules do not stain in anything but the plasma stain, and therefore cannot be considered as mitochondria. There are usually present, in the cytoplasm, several deeply staining bodies (hæmatoxylin) which are perhaps similar to the chromatoid corpuscles of other workers. It is possible that these may really be of a mitochondrial nature, but their subsequent fate cannot in any way be related to the mitochondria of the later stages. Lewis and Robertson (1916) in

their study of the living cells of *Chorthippus* (Orthoptera) found that mitochondria were present in the primary spermatogonia in the form of granular threads; but there is no evidence for such structures in *Passalus*.

The secondary spermatogonia are arranged in cysts in the form of a rosette, and are generally pyramidal in shape. Both nuclear and cytoplasmic volume is noticeably smaller in these than in the primary gonia. The cytoplasm always shows a marked affinity for the hæmatoxylin and this is due to the presence of numerous mitochondrial granules (Figs. 4, 23) which are scattered diffusely throughout the cytoplasm. Payne (1917) found granular mitochondria in the spermatogonia of *Gryllotalpa*, while Duesberg (1910) figures similar structures in *Blaps*. On the contrary Duesberg describes the mitochondria of the gonia of *Blatta* as being present in the form of threads (chondrioconts). Payne, Schäfer ('07) and others have found that the mitochondria of the spermatogonia are localized at the inner ends of the cells (*i. e.*, the end bordering on the cyst cavity). As I have before stated, in *Passalus* the mitochondria are diffusely spread and often are actually absent from the inner ends of the cells. Montgomery (1911) was unable to find evidence of "indubitable mitochondria" in the spermatogonia of *Euschistus*, although he found granules which he considered to be disintegrated idiozome material.

Fig. 5 shows a degenerating spermatogonium. It is noticed that the cytoplasm is much more deeply staining and the mitochondrial granules are much larger. The chromatin of the nucleus is concentrated into one or two karyosomes. The increase in the size of the mitochondrial granules is probably due to an agglutination of the smaller normal ones. Cowdry (1916) in his excellent review on the functional significance of mitochondria, has called attention to the relations of mitochondria in pathological tissues. He mentions the work of Scott who found that in fatty degeneration of the pancreas there was an agglutination of the mitochondria. It is quite possible that the degeneration of cells which is so common in insect spermatogenesis is of the fatty degeneration type. A study of the behavior of the mitochondria in degenerating sperm cells may throw some light on their rôle in normal processes. This is, however, beyond the scope of the present work.

The spermatogonial cysts which are in mitotic activity, stand out very clearly in contrast with the resting cysts. This is because of their lighter staining capacity; whether this is in turn due to the partial disappearance of the mitochondria, could not be ascertained (Figs. 2, 24). Buchner (1909) found that in *Gryllotalpa vulgaris* the mitochondria disappear during or just before cell-division. There are three possible explanations for the partial loss of mitochondrial structure during mitotic activity; (*a*) at this time the cytoplasmic volume is much greater and hence the mitochondria are more diffusely spread; (*b*) the dissolution of the nuclear membrane sets free a large amount of karyolymph which perhaps dilutes the cytoplasm and obscures the mitochondria; (*c*) the mitochondria may dissolve or become chemically changed so that they are no longer recognizable as such. At any rate, they soon appear in great numbers after cell-division, so that their partial disappearance was apparent and not real.

2. *Spermatocytes.*—In the spermatocytes at the beginning of the growth period, the mitochondria are still in the form of diffusely spread granules. There is a noticeable increase in their numbers as the growth period progresses, which plainly indicates that new ones are being formed (Fig. 6). Usually a denser perinuclear zone of cytoplasm can be seen during the growth period; the significance of this zone will be discussed later.

As the late prophase approaches, the cytoplasm becomes filled with numerous delicate threads deeply staining in hæmatoxylin. These are the filar mitochondria (chondrioconts) and they first appear at this time, although there is a slight indication of delicate granular threads in some of the earlier stages. The origin of these threads could not be traced, but it is quite likely that they are genetically related to the granules of the preceding stages. In the first spermatocyte the mitochondria appear diffusely spread when material has been fixed in Flemming's fluid (Figs. 7, 8, 25, 25*a*). In Benda and Hermann material the mitochondria agglomerate in dense irregular masses, still showing their filar nature (Figs. 9, 26). The threads tend to lie with their length extended in the direction of the main axis of the cell. Sections of the cell taken at right angles to its longitudinal axis show the mitochondria on end view (Fig. 25*a*). As the nuclear membrane dissolves and the first maturation spindle is forming (Figs. 10, 29), the mitochondria are usually localized at one side of the spindle. When the spindle has fully formed, the mitochondria begin to envelop it at first from one side (Fig. 11) and later entirely surround the outer spindle fibers, being equally distributed along their lengths (Figs. 13, 30, 31). By the time the metaphase has been reached, the spindle has been entirely surrounded by mitochondria. Figures 12, 32, 33, 34 represent cross-sections at different levels of the first maturation spindle at metaphase, showing clearly the relations of spindle and mitochondria. As is best seen in the anaphase (Figs. 13, 35) they do not entirely reach to the two poles of the spindle, so that there is a short uncovered part near the centrosomes.

The question arises as to the method of movement of the mitochondria in order that they establish this relation with the spindle; is it an active or a passive movement? Many observers on living cells have noted that the mitochondria are generally of a vibratile or motile nature. According to Lewis and Lewis (1914) the mitochondria are never at rest; "a single mitochondrium sometimes twists and turns rapidly as though attached at one end, like the lashing of a flagellum, then suddenly moves off to another position in the cytoplasm as though some tension had been released" (p. 332). If it is true that they possess the power of active movement, it may be possible in this way to explain their movement to the spindle. Lewis and Robertson (1916) state that "the mitochondria migrate from the two masses of mitochondrial granules and elongate towards the poles of the spindle," etc. (p. 108). Whether they mean by this an active or a passive migration is not clear from their account. It seems highly improbable that the movement is active. Some observers have supposed that their movement is influenced by the centrosomes, but there is no conclusive evidence that this is the case. It is more likely that the movement of the mitochondria to their position on the spindle is due to the cytoplasmic movements during metakinesis which Conklin has shown, plays such an important rôle in the localization of cytoplasmic substances in the egg.

There is no evidence from my observations on *Passalus* that the mitochondria divide autonomously, as has been maintained by some workers. As the cell constriction continues through the equator of the spindle (Figs. 14, 35), the mitochondria are divided by this constriction and the daughter cells (second spermatocytes) receive approximately equal amounts of mitochondria. The mitochondrial masses then move a short distance toward the poles; this is evidently caused by a further elongation of the spindle. Montgomery (1911) has described a similar method of division in *Euschistus*. The fact that mitochondria are found in equal amounts in daughter cells is supposed by some to favor the view that they divide autonomously; but it is well known that yolk and similar substances are often found in equal amounts in daughter cells, yet no one maintains that yolk granules divide autonomously. Fauré-Fremiet (1911) describes autonomous division in the mitochondria of the Protozoa, and Wilke (1912) does likewise for the spermatocytes of *Hydrometra*. Payne (1917) does not incline strongly to the view that the mitochondria are divided by the cell constriction, while Lewis and Robertson (1916) do not clearly state whether their division in *Chorthippus* is autonomous or passive.

Following the first maturation division, there is a short interkinesis during which the second maturation spindle is formed (Figs. 16, 36). At this time the mitochondria are lying at one side in a rather dense mass which shows a lighter central portion through which the spindle of the previous division passed. The behavior of the mitochondria during the second maturation division is precisely the same as in the first division (Figs. 17, 37). They again surround the spindle peripherally and are divided by the equatorial constriction. As a result of this division both spermatids receive approximately equal amounts of mitochondria (Fig. 18).

The mitochondrial mass contained in the spermatid gradually becomes more compact and henceforth may be designated as the Nebenkern. It stains intensely with the basic stains and often shows a granular structure. It often shows a lighter central portion which represents the position the spindle had occupied (Fig. 20*d*). Soon after the Nebenkern has become a spherical body, it begins to show a peripheral lighter area which is of a vacuolar nature (Fig. 19). As the spermatid elongates the Nebenkern becomes elongated and the vacuolization of the peripheral layer becomes more marked (Fig. 20, *a*, *b*). It becomes divided into two halves and is later pierced by the axial filament (Figs. 21, 39). As the axial filament grows out, the Nebenkern continues to elongate forming a sheath about it. The material of the Nebenkern thus extends from the centrosome for a considerable distance along the axial filament (Fig. 22). Fig. 20, *c* is that of a cross-section through the tail of a spermatid showing the two halves of the Nebenkern on each side of the axial filament. Whether or not the Nebenkern sheath extends to the free end of the axial filament could not be determined. As the transformation continues, the Nebenkern becomes more lightly staining, and all evidences of its mitochondrial nature are lost.

3. *Discussion.*—I have been unable to obtain any definite and conclusive evidence as to the manner in which the mitochondria arise. There can be no doubt that they increase in numbers during the growth period, and the question arises: to what influence do the mitochondria owe their origin? This has been and still is a much-debated cytological question, and much depends on the final solution of the problem.

Meves, Bouin, Duesberg and others have always maintained that the mitochondria are persistent and self-perpetuating structures, much as the chromosomes of the nucleus are at present regarded. On the other hand, Goldschmidt and his pupils, Buchner, Jordan, Wildman and others have derived the mitochondria from the nucleus, and have thus maintained that they are akin to the chromidia of Hertwig. Vejdovský has traced their origin from the sphere material, while Montgomery in *Euschistus* concludes that they are probably derived from the idiozome or the nucleus, or by a "joint action" of both. In their studies on the living sperm cells of *Chorthippus*, Lewis and Robertson are unable to find any evidence that the mitochondria are derived from the nucleus. It is thus evident that the facts are conflicting on all sides. If it is true, as I have previously stated (p. 408), that the mitochondria are not present in the primary spermatogonia of *Passalus*, then we cannot maintain that they are persistent and self-perpetuating. I am, however, not entirely convinced that they are absent in the primary spermatogonia and shall await fresh material for a study of the living cells.

It is possible that the increase in number of the mitochondria during the growth period is due to a simple growth and division of those already present. I cannot find any evidence for this in my material. In nearly all the growth stages of the first spermatocytes, there is present a denser and more deeply staining perinuclear zone. Schäfer (1907) has figured similar conditions in *Dytiscus*, while Voivnov (1903) shows it more strikingly in *Cybister* and calls it the "zona interna." During the maturation divisions, the "zona interna" surrounds the spindle peripherally and forms the Nebenkern of the spermatid. As Duesberg (1910) has pointed out, it is quite evident from this behavior that the "zona interna" is really of a mitochondrial nature. Giardina (1904) has studied the oöcytes of a number of forms (*Periplaneta*, *Stenobothrus*, *Gryllus*, *Mantis*, etc.) and figures well-defined perinuclear zones usually of a granular nature. Whether this zone is formed by a diffusion of chromatin in the form of a solution out of the nucleus into the surrounding cytoplasm or whether it arises in situ in the cytoplasm by interaction with the nucleus, is a question which Giardina discusses at considerable length. He concludes in favor of the latter view. Payne (1917) has described a similar perinuclear zone in the oöcytes of *Gryllotalpa*, but he definitely states that this consists of mitochondria. As the oöcyte grows, the mitochondria migrate centrifugally into the cytoplasm. Vejdovský (1911–12) shows the mitochondria arranged in a definite perinuclear zone in the spermatocytes of *Diestramena*. It thus seems quite probable that the perinuclear zones described by Giardina are really of a mitochondrial nature. The occurrence of this zone in such close connection with the nucleus and especially during a period when the mitochondria are certainly increasing in number, is highly indicative of an interaction between nucleus and cytoplasm. The relation of this zone to the mitochondria seems to be a strong argument, at least, that it is the locus of mitochondria formation.[1]

The association of the spindle and the mitochondria has led to some confusion in regard to the origin of the Nebenkern, so that some workers have ascribed its origin to the spindle remains of the second maturation division. Arnold (1908) comes to this conclusion in his study of *Hydrophilus*, as does Baumgartner in *Gryllus*. Voivnov, as previously mentioned, derived the Nebenkern from his "zona interna" and the peripheral spindle fibers. Munson (1906) in his work on *Papilio* also derives the Nebenkern from the spindle remains, but his observations are interesting since they clearly show that he has confused these with the mitochondria. According to his view, it is only the "outer, granular" mantle fibers of the spindle which take part in the formation of the Nebenkern. It is evident, in light of the recent work, that the "outer, granular" mantle fibers are really mitochondria. That he actually saw the mitochondria and misinterpreted them, is evident from the following quotation: "Often a few scattered chromatin segments are found scattered along the spindle fibers, or else drawn out into stainable threads parallel with the spindle fibers" (p. 91).

(*c*) *Spindle Derivatives.*

When one attempts to review the literature on the subject of spindle derivatives and their histories, one is immediately confronted with a maze of conflicting observations and interpretations, to say nothing of a nomenclature which is almost hopelessly confused. The names mitosome, idiozome, attraction-sphere, centrosphere, astrosphere, Nebenkern (of older workers), etc., are all examples of the existing confusion, and should caution us against hasty interpretations of such structures. Meves (1899) states that he first applied the term "idiozome" to those compact bodies in the spermatogonia and spermatocytes which surround the central corpuscles (centrosomes). By a disintegration of the idiozome, the centrosomes are set free and then take part in the next cell-division. In the rat, however, Meves found that there is no disintegration of the idiozome, but that the centrosomes wander out, leaving the idiozome intact. As division progresses, the latter dissolves and disappears. There is no essential difference between the "attraction-sphere" of Van Beneden, the "centrosphere" of Strasburger and the "astrosphere" of Fol and Boveri; and so far as I have been able to ascertain, there is no fundamental difference between these last named structures and the "idiozome" of Meves. One thing is clear,—that these structures all refer to the achromatic substance of the spindle situated at the poles and usually enclosing the central corpuscles. To avoid any possible misuse of these terms I shall employ the non-committal term "sphere" to denote this portion of the spindle. With regard to the remains of the spindle proper, there is less confusion of terms, and I shall use the term "mitosome" or "spindle remains" to designate this structure.

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[1] It is interesting to note in this connection that the material of the yellow crescent in the ascidian egg has been shown by Conklin to be found at various times in a perinuclear position; and Duesberg (1913) has shown that this zone is extremely rich in mitochondria.

1. *Spermatogonia.*—The spermatogonia in *Passalus* in mitotic activity stand out very sharply in contrast with those in the resting condition not only because of their lighter staining capacity (as mentioned on p. 410), but also because they tend to become round in outline and the cell walls become more sharply defined. This is indicative of an internal pressure which Reinke (1900) calls the "mitotic pressure." As the gonial anaphase progresses, a well-defined cell-plate makes its appearance and stains deeply in hæmatoxylin (Figs. 2, 24). As the telophase advances, the spindle and cell-plate become more compact, the former taking the acid stains and the latter taking the basic stains. Fig. 4 shows several resting cells from a spermatogonial cyst with the spindle remains (mitosome) running from cell to cell. Very often the spindle remains from each division become so connected that they form sort of bond between all the cells in a cyst. Hegner (1914) has described a similar condition in the spermatogonial cysts of *Leptinotarsa decemlineata.* In this case, however, he found that material fixed in Carnoy's fluid always showed the spindle remains taking the basic stain.

Günthert (1910) has described similar results in the differentiation of nurse cells and oöcytes in *Dytiscus* and Govaerts (1913) has done likewise in *Carabus*. In *Passalus*, it is only in the region of the cell-plate that the spindle takes the basic stain. In cross-section the spindle remains appear as plasmasome-like bodies in the cytoplasm. Often there may be seen deeply staining granules adhering to the surface of such bodies; these I take to be portions of the deeply-staining cell-plate.

2. *Spermatocytes.*—The spindle remains of the last spermatogonial division (together with the cell-plate) persist into the spermatocyte and remain very conspicuous throughout the entire growth period (Figs. 6, 8, 27). Every spermatocyte exhibits these spindle remains, either in their original positions connecting cells or else in the form of plasmasome-like bodies lying free in the cytoplasm. Similar conditions have been shown by Voivnov in *Cybister* and by Munson in *Papilio*. In *Gryllotalpa*, Payne states that "there is no indication that a sphere or the spindle and astral fibers persist after cell-division." In his figure B, plate 1, he shows two plasmasome-like bodies present in the cytoplasm of a spermatocyte during the early growth period. As to the origin of these bodies, he is uncertain, but he is not ready to admit that they may be idiozome material. I agree that it would be rather speculative to assign the term "idiozome" to these bodies, but in *Passalus* it is quite clear that similar bodies are derived from the spindle. I am not able to establish the presence of a definite "idiozome" or sphere material in the spermatocytes of *Passalus* and just where the centrosomes lie hidden during the growth period is a difficult matter to determine. Since the cells are so filled with mitochondria at this time, it seems almost impossible to definitely locate them. The centrosomes are first discernible when the first maturation spindle makes its appearance. From the long persistence of the spindle remains of the last spermatogonial division, it may be concluded that its substance must be of some inert, resistant material. Munson (1906, p. 90) makes the following remarks on *Papilio:*

"The resistance of the maturation spindles to reagents is remarkable. In studying the living dividing cells on the slide, I have seen the cytoplasm gradually disintegrate, become vacuolated, and disappear while the spindles remain as perfect as ever. In testing the effects of reagents, too, I have been able to dissolve practically the whole of the cytoplasm of all the cells of the cyst, while the spindles remained, showing a system of connected spindles throughout the whole cyst."

There are other structures related to the spermatocytes which are of a more or less problematical nature. In Figs. 8 and 28 there will be noticed a large vacuole in each of the spermatocytes; these are to be found in nearly every spermatocyte during the late prophases and thus far I have been unable to find them in the earlier stages. The vacuoles are filled with a homogeneous fluid (perhaps a gel) which takes the plasma stain but lightly. Sometimes there may be several vacuoles in a single cell. In cysts of spermatocytes having such vacuoles, one often finds in the cyst cavities round or oval bodies of a granular nature (Fig. 8) resembling very much the cytoplasm of the spermatocytes. Furthermore, in such cysts the sides of the spermatocytes bordering on the cyst cavity are of an irregular outline and often distinct pseudopod-like projections of the cytoplasm are given off. It is therefore highly probable that the small bodies found free in the cyst cavity have arisen from the pseudopod-like projections or buddings from the cytoplasm of the spermatocytes. What the significance of either the vacuoles mentioned above, or of the casting off of portions of the cytoplasm may be, I am unprepared to state. The presence of vacuoles and pseudopods at the same time may indicate that the vacuoles are cast out by means of setting free the pseudopods. But this view is at present untenable, for I have never actually seen vacuoles within the pseudopods; furthermore the contents of the vacuoles are always of a homogeneous appearance, while the cast off parts of the cytoplasm are granular as described above. Voivnov has found in *Cybister* that vacuoles make their appearance in the late prophases of the spermatocytes. These differ in appearances, at least, from those found in *Passalus* in that they contain definite bodies as inclusions. Voivnov also finds that they are later cast out into the cyst cavity. He considers these to be portions of the sphere (idiozome) which are undergoing disintegration. In support of this view, he finds that they appear at a time when the centrosomes are first set free and often they may be found in close spatial relations to the centrosomes.

Whether or not the vacuoles and the cast-off portions of the cytoplasm in *Passalus* correspond to the conditions described by Voivnov in *Cybister* I am at present unable to say. The origin of the vacuoles and their relation to the cytoplasmic buddings can be best studied in the living material, and I shall leave the question undecided here for lack of evidence.

3. *Maturation Divisions and the Spermatid.*—The spindles developed for both maturation divisions are relatively very large, both in actual dimensions and in number of spindle fibers. Correlated with the large size of the spindles is the relatively large nucleus, containing little chromatin but a large amount of karyolymph. The view of Conklin and others, that the spindle grows at the expense of the karyolymph is certainly substantiated in the case of *Passalus*. In both maturation divisions cell-plates are developed which are very much smaller than those found in the spermatogonia (Figs. 14, 8), and the remains of the spindles persist for a considerable time after division. In the anaphase of the second maturation division, the centrosomes are still distinguishable lying close to the chromatin masses at the poles. In the telophase of this division they are found lying on the nuclear membrane. It is because of this position on the nuclear membrane that they are not easily detected, but careful searching and focusing will show their undoubted presence (Fig. 18). Thus in the spermatid, the centrosome is still to be seen closely attached to the nuclear membrane; it gradually shifts its position until it comes to lie between the nucleus and the Nebenkern. Usually there is a precocious growth of the axial filament (Fig. 19) before the centrosome arrives at its ultimate position. At this time, the centrosome appears double, while the axial filament grows out between the two halves of the Nebenkern and becomes associated with the latter in the formation of the tail, as shown before (p. 413). As transformation continues (Figs. 21, 22), the centrosomes become more and more closely associated with the nucleus, until in the older stages they are scarcely

distinguishable from the head of the spermatozoön, and hence there is no well-defined middle-piece.

In the youngest spermatid there is always present a cytoplasmic body of a refringent nature which takes the plasma stain (Figs. 19, 38). One portion of it is more compact, while the other part is in the form of a vacuole containing a deeply staining (hæmatoxylin) body. This structure is undoubtedly a spindle derivative, but whether it represents the sphere material or the mitosome I am unable to state. As to the origin of the deeply staining body contained within it, I am equally unable to explain. It may possibly represent a portion of the cell-plate which has become detached from the cell wall and has become encompassed in the remains of the spindle.[1] In older spermatids a compact portion of the spindle derivative is found lying near the nucleus in that part of the spermatid which is destined to give rise to the head end of the spermatozoön (Fig. 21). In later stages, as the nucleus becomes laterally compressed, this structure is transformed into the acrosome. The remainder of the spindle derivative is sloughed off into the tail of the spermatozoön and gradually disappears.

4. *Discussion.*—As has been previously stated, the axial filament in *Passalus* arises in connection with the centrosome. Munson (1906) has expressed an entirely different view concerning its origin in *Papilio.* His view is that the axial filament represents a much compressed portion of the cytoreticulum and has no relation to the centrosome. Accordingly, he finds that in the stages of development of the axial filament occasionally three or four filaments may be present in a single spermatid, but these finally unite into a single thread. Paulmier (1899) has described double and quadruple spermatids in which two or four axial filaments were present, but each was connected to a centrosome. Munson's view arises from the fact that he has assigned a wholly different function to the centrosome. His figures of the spermatids of *Papilio* show spindle derivatives each containing a deeply staining body which he interprets as

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[1] Duesberg (1908) figures a similar spindle derivative in the spermatid of the rat which he designates as the "idiozome." It also consists of a vacuolar portion which contains a deeply-staining granule which is not a centrosome, just as in *Passalus.*

the centrosome. The conditions here are very much like those in *Passalus* (Figs. 19, 38), but in the latter case the deeply staining body is certainly not a centrosome. The sphere (?) of the spermatid of *Passalus* has no connection with the centrosome. This is in agreement with Montgomery's conclusions in *Euschistus*. According to Munson, the centrosome gives rise to the acrosome of the spermatozoön.

Payne (1917) has approached the subject of spermatid transformation with more or less scepticism as to the generally accepted origins of the structures present here. I am in hearty accord with this point of view, but it seems that Payne has carried matters too far. I agree that to call the structure from which the axial filament grows a centrosome without tracing its history from the second spermatocyte, is highly speculative. But to say, as Payne does, that at a certain stage of the spermatid "there is nothing in the cytoplasm but mitochondria" (p. 309) is equally as dogmatic and unwarranted in light of the observations of many other workers. In *Passalus* there can be no doubt that the centrosome of the spermatid has actually been carried over from the preceding cell-division. Furthermore, in the youngest spermatid there can always be found the refringent cytoplasmic body which is undoubtedly a spindle derivative. In *Gryllotalpa*, Payne finds in the stages succeeding the young spermatids which contain no other cytoplasmic structures but mitochondria, the sudden appearance of two deeply staining bodies, one of which later forms the acrosome, while the other is pushed off into the tail. Since these were not present in the earlier stages, they have apparently arisen "de novo," that is, they are newly differentiated parts of the cytoplasm. One might then expect to find developmental stages of such structures, but Payne does not indicate such. However, it seems to me that the building-up of the spermatozoön from the spermatid is a process involving no differentiation, but a *transformation* of differentiations already present. All the structures needed in the building up of the spermatozoön are at hand, and there is no further elaboration of new ones. Munson (1906, page 96) has clearly expressed a similar view:

"The comparative inertness of the nucleus at the close of the last maturation division and ever afterward would not justify us in assuming that this is a growth period in the history of the spermatozoön; but rather that it is a transformation period of those organs that are already present and fully-grown in the spermatid stage. This transformation in all the organs of the cell is merely an elongation such as could be brought about, doubtless, by prolonged lateral pressure."

D. GENERAL CONSIDERATIONS.

The importance of the mitochondria as bearers of hereditary units rests on their mode of origin and maintenance through the cell cycle, their behavior in fertilization and their rôle in differentiation. If, as has been previously discussed, the view is correct that the mitochondria owe their origin to materials derived from the nucleus or by the activity of the nucleus, then their importance in heredity can only be secondary.

Just how much of the spermatozoön enters the egg is a matter of importance in ascertaining the rôle of the mitochondria. In *Nereis*, Lillie (1912) has found that the middle piece and the tail of the spermatozoön do not enter the egg. On the other hand, Meves (1911) has shown that the entire spermatozoön enters the egg of *Ascaris*, and Van der Stricht (1909) has shown similar results in the bat. It therefore seems impossible to make any generalizations on this subject until more work on the details of fertilization has been done. In the case of *Peripatus*, Montgomery (1912) has shown that the mitochondria are entirely lost in the spermatozoön, being thrown off within certain cytoplasmic lobes. Here, at least, the mitochondria of the spermatozoön can play no part in the transmission of hereditary characters.

The mitochondria have been most exhaustively studied in somatic cells where they are present in a variety of forms. Certain workers (Meves, Duesberg, Hoven and others) have maintained that the mitochondria give rise to myofibrils, neurofibrils and other somatic differentiations; but these views have not been strongly substantiated. The work of Cowdry (1914) is strong evidence that the mitochondria of nerve cells are not transformed into neurofibrils.

The researches on the chemistry of mitochondria are practically all in agreement that they are phospholipins or lecithin-albumins. No one has attempted to show that yolk is a bearer of hereditary units; yet yolk is chemically allied to mitochondria. In fact Fauré-Fremiet has shown that mitochondria actually transform into yolk. The work on the chemistry of mitochondria indicates that they are of great importance in the metabolic activity of cells, but our knowledge of their relation to heredity is negative. To say that the mitochondria are not the bearers of hereditary units is not denying that they may *influence* heredity in some cases, just as we know that heredity can sometimes be influenced by environmental conditions of food, temperature, etc.

One fact which has come clearly to light from this study is that beginning with the spermatogonia and continuing up to the spermatid, there is a progressive elaboration of mitochondria. They are then transformed into a definite structural part of the spermatozoön, the sheath of the axial filament. This progressive increase in the amount of mitochondria seems to indicate that they are differentiation products. Hence if there is any genetic continuity between the mitochondria of successive cell generations, it is only of a limited sort. The conception that the mitochondria present in the somatic cells are the direct descendants of those of the germ cells from which they have arisen, certainly has very little evidence in its favor. It seems more probable that mitochondria are in the nature of cytoplasmic differentiations, akin to metaplasm (yolk, etc.) and without a definite relation to the development of hereditary characters, but with the capabilities of influencing development insofar as they may be related to the metabolic activity of cells. It is possible that in the spermatozoa, the mitochondria merely function as locomotory organs.

SUMMARY.

1. Although mitochondria can not be definitely demonstrated in the primary spermatogonia of *Passalus cornutus*, they are present in the secondary spermatogonia in the form of numerous and diffusely spread granules.

2. The mitochondria increase in number during the growth

period, and in the later stages are found in the form of threads (chondrioconts) which lie in the direction of the chief axis of the cell.

3. During the maturation divisions, the mitochondria envelop the spindle peripherally and are divided by the cell constrictions, so that daughter cells receive approximately equal amounts.

4. The mitochondria of the spermatid form the Nebenkern, which later is pierced by the axial filament. As the latter grows, the Nebenkern elongates, forming a sheath about it.

5. Spindle remains are found forming connections between the spermatogonia. The spindle remains of the last spermatogonial division persist throughout the entire growth period of the spermatocyte.

6. A spindle derivative is found in the spermatid, a portion of which gives rise to the acrosome of the spermatozoön.

7. The centrosome of the second maturation division is carried into the spermatid and gives rise to the axial filament of the spermatozoön. The centrosome becomes so closely associated with the nucleus that there is no well-defined middle-piece in the spermatozoön.

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ABBREVIATIONS.

c., centrosome; *m.*, mitochondria; *N.*, Nebenkern; *s.*, spindle derivative.

All drawings were made with the aid of a camera lucida at table level using a Zeiss 12 ocular and a 2 mm. oil objective. The reproductions have been reduced one-third.

EXPLANATION OF PLATE I.

FIG. 1. Primary spermatogonium. No clear indication of the presence of mitochondria. Note small mass of chromatoid substance in cytoplnsm.

FIG. 2. Anaphase of secondary spermatogonium. Note lightly-staining cytoplasm, well-defined cell-plate, and the tendency of the cell to become round.

FIG. 3. Metaphase plate of ovarian follicle cell, showing thirteen equal pairs of chromosomes.

FIG. 4. Resting stages of secondary spermatogonia. Mitochondria granular and diffusely spread. Note persistence of spindle and cell-plate.

FIG. 5. Degenerating spermatogonium. Mitochondria larger in size; chromatin concentrated in two large karyosomes.

FIG. 6. Pachytene stage of first spermatocyte. Mitochondria still granular, with only a slight indication of threads forming; note denser perinuclear zone and the persistence of the spindle remains of the last spermatogonial division.

FIG. 7. Late prophase of first spermatocyte, Flemming fixation. Nucleus uncut. Mitochondria filar, with their lengths in the direction of the chief cell axis.

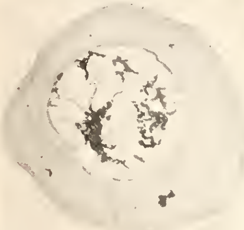
FIG. 8. Similar stage as above, showing remains of spindle still persisting, two large cytoplasmic vacuoles of problematic origin, and small bodies in the cyst cavity which have been budded off from the cytoplasm of the spermatocytes.

FIG. 9. Prophase of first spermatocyte, Hermann fixation; showing agglomeration of the mitochondria.

FIG. 10. Formation of the first maturation spindle; mitochondria at one side.

FIG. 11. Mitochondria beginning to envelop first maturation spindle.

FIG. 12. Cross-section of the metaphase plate of first spermatocyte, showing mitochondria completely surrounding the spindle. Thirteen bivalent chromosomes.



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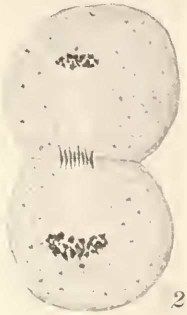

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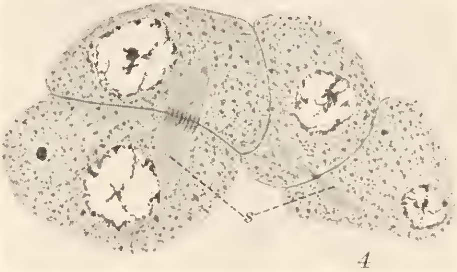

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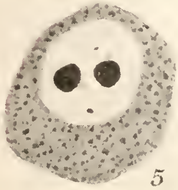

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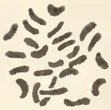

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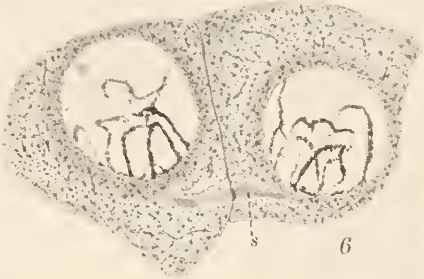

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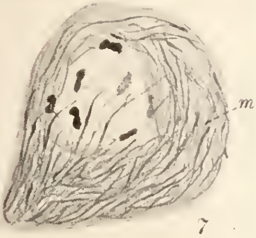

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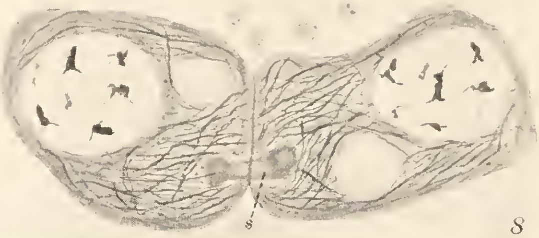

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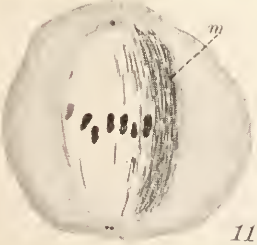

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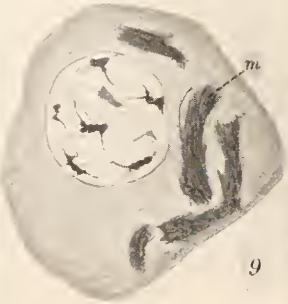

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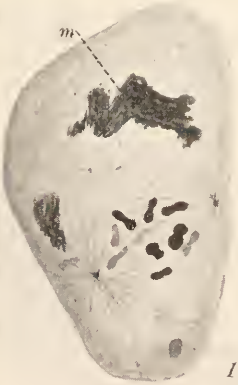

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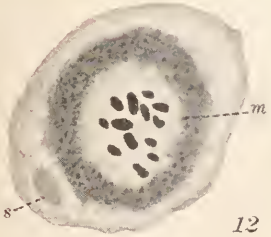

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EXPLANATION OF PLATE II.

FIG. 13. Anaphase of first spermatocyte, showing relation of spindle and mitochondria.

FIG. 14. Late anaphase of first spermatocyte. Cell constriction has divided mitochondria so that the daughter cells contain approximately equal amounts.

FIG. 15. Cross-section of first spermatocyte near pole of spindle, showing absence of mitochondria here.

FIG. 16. Second spermatocytes (interkinesis). Mitochondria present in daughter cells in compact masses, also showing the persistence of the spindle and cell-plate.

FIG. 17. Metaphase of second spermatocyte. Mitochondria again surrounding the spindle peripherally.

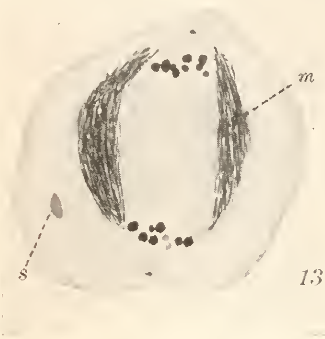
FIG. 18. Telophase of second maturation. Mitochondria again divided by the cell constriction into two equal masses. Note position of the centrosome (*c*) lying on the nuclear membrane.

FIG. 19. Spermatid. Nebenkern (*N*) derived from the mitochondria, with a peripheral vacuolated portion. Persistence of centrosome (*c*) with a precocious growth of the axial filament; spindle derivative (*s*) enclosing a deeply-staining body.

FIG. 20. Changes in the Nebenkern during transformation of spermatid. *c*, cross-section of the tail showing the Nebenkern lying on each side of the axial filament. *d*, Nebenkern with lighter central portion where spindle of previous division had passed through.

FIG. 21. Stage in the transformation of the spermatid. A portion of the spindle derivative (*s*) occupies a position at the head end and a portion passes into the tail. Nebenkern elongating, and the axial filament growing out of the centrosome (*c*) between the two halves of the Nebenkern.

FIG. 22. Later stages in the transformation of the spermatid. *b*, giant spermatid.



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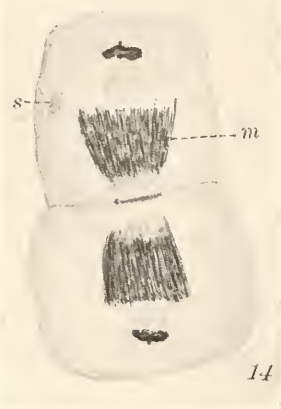

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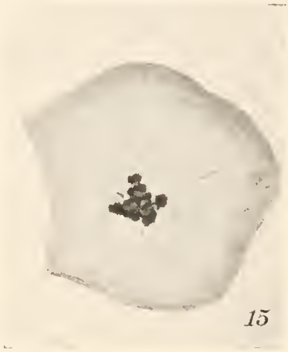

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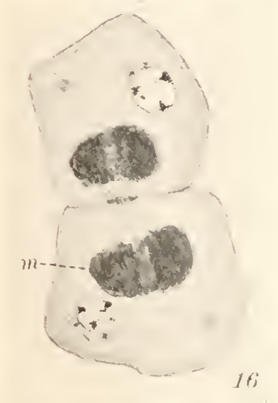

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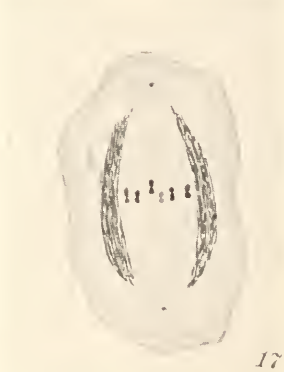

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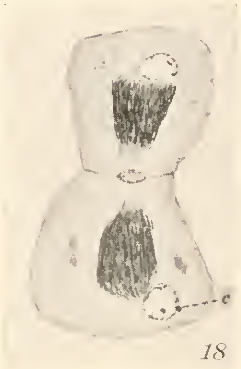

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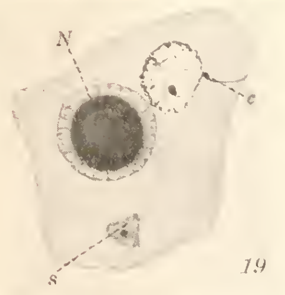

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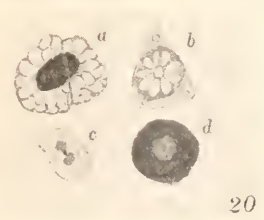

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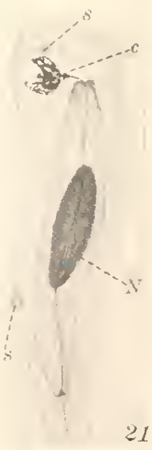

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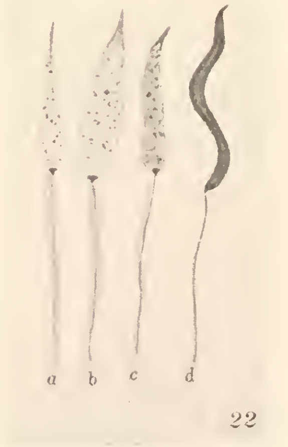

22

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EXPLANATION OF PLATE III.

Photomicrographs taken at a magnification of about 1,500 diameters

FIG. 23. Resting secondary spermatogonia, showing diffuse granular mitochondria deeply staining in iron-hæmatoxylin.

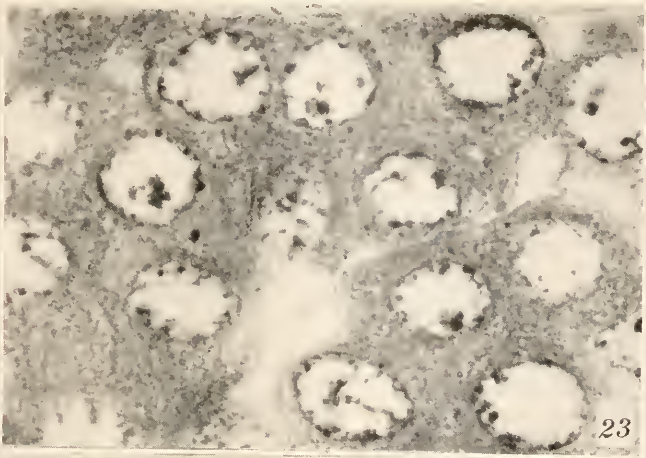
FIG. 24. Telophase of a secondary spermatogonium, showing the deeply staining cell-plate. The rest of the spindle here shows somewhat darker than it really is; it always takes the plasma stain.

FIG. 25. Late prophases of first spermatocyte; Flemming fixation. The mitochondria are filar, with their lengths extended in the direction of the chief axis of the cell; the nuclei here are uncut and somewhat out of focus. Fig. 25*a* is a section at right angles to the chief axis of the cell and shows the mitochondria on end view.

FIG. 26. First spermatocyte from a Hermann fixation. The mitochondria are agglomerated in dense masses (*m*).

FIG. 27. Part of a cyst of first spermatocytes to show the spindle remains (*s*) forming a connection between several cells; parts of it stain deeply with iron-hæmatoxylin.

FIG. 28. First spermatocyte greatly destained to show the cytoplasmic vacuole of unknown significance.



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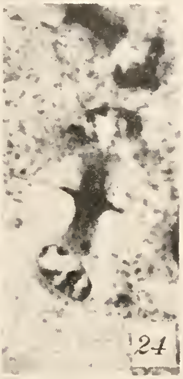

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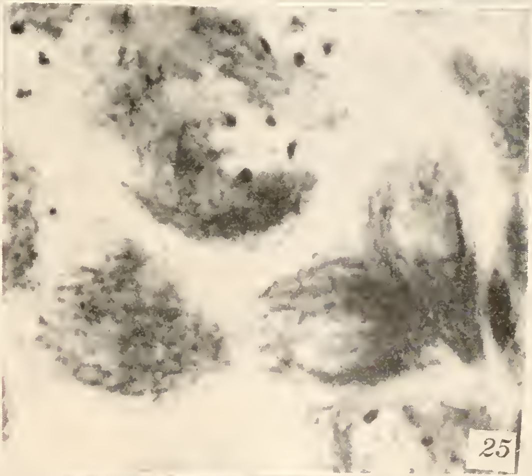

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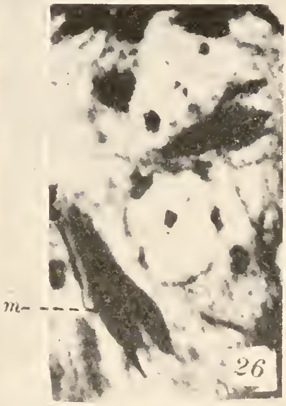

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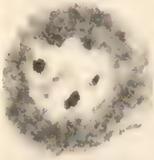

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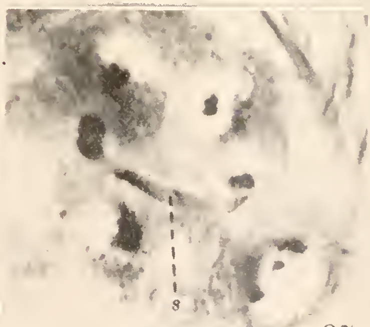

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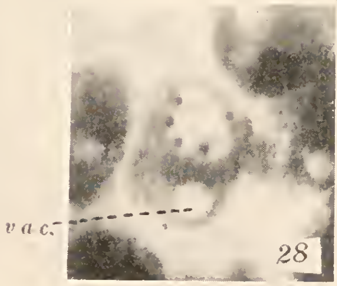

28

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EXPLANATION OF PLATE IV.

FIG. 29. Formation of first maturation spindle; the mitochondria are at one side and are beginning to envelop the spindle.

FIG. 30. Metaphases of first maturation division; the mitochondria completely surround the spindle.

FIGS. 32, 33, 34. Cross-sections of the first maturation spindle, showing its relation to the mitochondria.

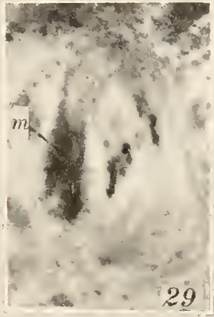
FIG. 35. Late anaphases of the first maturation division; the mitochondria are densely staining and closely applied to the spindle. The mitochondria do not reach to the poles.

FIG. 36. Second spermatocyte; interkinesis. Mitochondria in a dense mass awaiting the formation of the second maturation spindle.

FIG. 37. Second maturation spindle forming with the mitochondria at one side.

FIG. 38. Young spermatid with deeply staining Nebenkern (*N*) lying close to the nucleus, and the spindle derivative (*s*) which contains a deeply staining corpuscle. Centrosome not clearly in focus.

FIG. 39. Later stage in spermatid transformation, showing the elongation of the Nebenkern after the axial filament has grown out.



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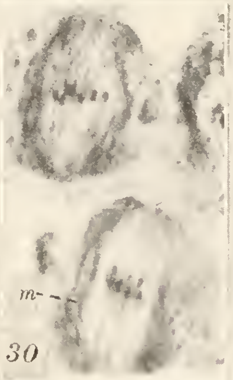

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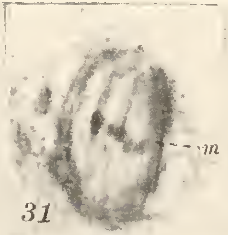

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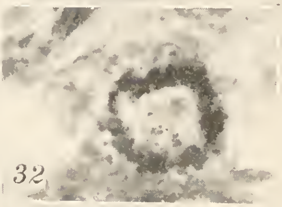

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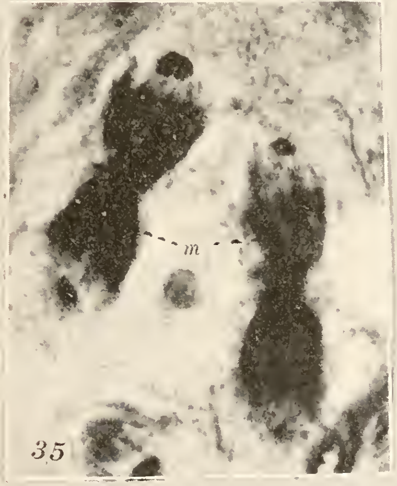

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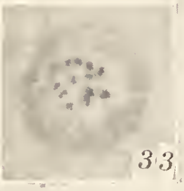

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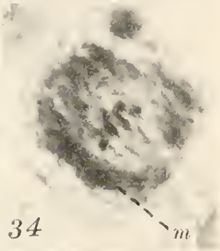

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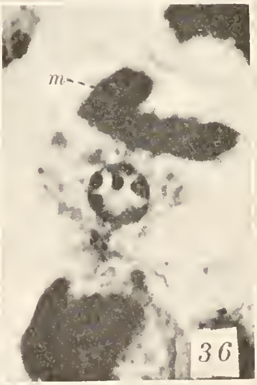

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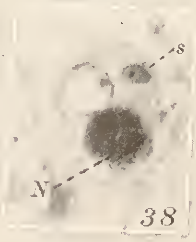

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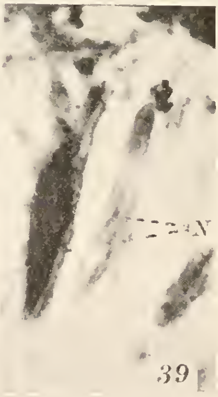

39

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